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Symposium on Chemistry, Biochemistry and Metabolism of Lipids*

THE day of meetings is with us. Small conferences become large meetings. Large meetings become colossal productions, some of them with considerable in the way of Hollywood trimmings.

In the belief that the time for a reverse swing of the pendulum was more than due, several years ago a small group of men, including the late Harry Deuel, Wendell Griffith, Sidney Madden, and the undersigned decided that it would be well to institute a Pacific Coast Metabolic Conference which would have only one adamant rule, namely, that it would never be permitted to become large.

The 1957 and 1958 Conferences have been devoted to the subject of lipids. Because of the great contributions of Harry Deuel to the understanding of lipid biochemistry and metabolism, in 1958 it was decided to name this conference and future conferences "The Deuel Conferences on Lipids."

Because of the large amount of material included in the presentations and in the discussion, only a portion of the Conference Proceedings is included in this issue of THE AMERICAN JOURNAL OF CLINICAL NUTRITION. It is probable that discussion will be included in the transcript of future conferences.

Perhaps the most important single contribution to the 1958 Conference was that of Dr. James and his colleagues. The gas phase chromatograph devised by James and Martin promises to clarify great areas which until the present time have been shrouded in ignorance. There is reason to believe that the use of this instrument plus the chromatographic column for separation of the lipid fractions of plasma and tissues will aid materially in the understanding of lipid metabolism in general and in the solution of the problems of atherogenesis in particular.

To those responsible for the arrangements, for this, the first of the Deuel Conferences, it seems most probable that the 20 years ahead will be most fruitful years in the field of clinical nutrition. It is for that reason that they, together with the Editors, decided to publish this Symposium in THE AMERICAN JOURNAL OF CLINICAL NUTRITION.

Perusal of Dr. Sinclair's poetic masterpiece will make it apparent that whatever the shortcomings of the Symposium, at least it was not stuffy. Dr. Sinclair's contributions to the discussion, particularly in the field of essential fatty acids, were as stimulating as his poetry.

In the papers which follow, more questions are raised than are answered. It seems probable that contributions to these and other lipid conferences in the years immediately ahead will supply many of the answers.

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Hiawatha's Lipid

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From his briefcase Hiawatha
Took his paper for the meeting,
Typed in triple-spacing and in
Triplicate on foolscap paper;
Glanced upon the crowd before him,
Critical and very hostile,
Like the lions in the arena
Waiting for a Christian victim,
As the surgeons in some theatre
Wait impatiently the patient;
Saw them with their notebooks waiting,
Saw the tape-recorder ready
Gleaming in its chromium-plating
By the Ampex Corporation
And preserving all the nonsense
Spoken by the previous speakers,
As the snow upon the prairies
Uselessly records the footprints,
So preserving all the nonsense
Spoken by the previous speakers.

INTRODUCTION

Hiawatha, taking courage,
Started on the Introduction,
Giving first a brief description
Of the Proto-Keysian period
When all fats in equal measure
Raised cholesterol in serum:
Butter, sardines, walrus liver,
Margarine or safflower seed oil,
Or arachidonic acid,
Or the body-fat of quokkas,
Or adrenals of the musk-rat,
Or the milk of female reindeer—
All these fats in equal measure
Raise cholesterol in serum,
As the rain in San Francisco
Fills the ditches in the roadways
(So at least thought Hiawatha)
So these fats in equal measure
Raise cholesterol in serum.

Then the Meso-Keysian period
When it's known from work of others
Quantitative variations
Do occur when different lipids
Are included in our diets,
Plentifully in our diets:
Butter, sardines, walrus liver
Margarine or safflower seed oil,
Or arachidonic acid,
Or the body-fat of quokkas,
Or adrenals of the musk-rat,
Or the milk of female reindeer—
Do not in an equal measure
Raise cholesterol in serum;
As the smoke is wind-swept upwards
Randomly with Brownian movement
Wandering above the wigwam,
So these fats in different measure
Raise cholesterol in serum.

Then the Neo-Keysian period
When arithmetic will tell us
By an intricate equation
What cholesterol in serum
We will have when we have eaten
(And don't vomit having eaten)
Butter, sardines, walrus liver,
Margarine or safflower seed oil,
Or arachidonic acid.
Or the body-fat of quokkas,
Or adrenals of the musk-rat,
Or the milk of female reindeer;
Count the double bonds and add by
Electronic automation
On a digital computer
From the Ampex Corporation;
There's no need to estimate it—
All cholesterol in serum
Follows now the Keys equation;
As the caribou in summer
Migrate by accustomed pathways
And predictably are herded,
Dietetic computation
Of the double bonds in lipids
With a slide-rule calculation
Gives you now a neat prognosis
Whether you will die tomorrow
From a thrombus in your vessels—
Myocardial infarction
Or ischaemic heart diseases—
As a cork pushed in a bottle
Stops the wine from flowing freely
(Vin Rose of California);
Atheromatosis also
Is predicted by this method,
By this skillful Keysian method.

Others are not quite so lucky:
Larry's lowered lipid levels*
After vegetable seed oils—
Polyethenoic acids
Or essential fatty acids
From the vegetable seed oils—
Follow a more simple pattern,
So at least thought Hiawatha
In unpublished observations:
As the sun comes up in morning,
As the sun goes down in evening,
So the laws of lipid levels
Are predictably determined:
Saturated fatty acids
Raise cholesterol in serum,
Polyethenoic acids
Lower serum lipid levels—
So at least thought Hiawatha
In unpublished observations.

METHODOLOGY

After this review of others
Hiawatha turned to methods
(Methodology, he called it
Making it more scientific—
Longer words are scientific);
Talked about silicic acid,
Mead's silicic acid column,
How he trapped the different lipids
As he used to trap the beaver.
Then he pushed them back and forwards—
Countercurrent distribution—
As the frightened hare or reindeer
Runs at random back and forwards.
Then he boiled them up with potash,
Alcoholic potash mixture,
Following the rules established—
Riemenschneider's "Skillful witchcraft"
So politely called by Mattson
(Personal communication)—
As the dinner in the stewpot
Is boiled by Minnehaha,
So he boiled them up with potash
And the double bonds determined
Spectrophotometrically.
Then he used the latest method,
Gas-chromatographic method
Introduced by James and Martin,
Showing peaks upon the paper
Like the Rockies at the sunset,
Like the mole-hills in the prairies.
Thus he estimated lipids
And he wondered if it mattered,
Wondered secretly about it
With unpublishable wond'rings.

RESULTS

Thus supplied with diverse methods
Hiawatha took some serum
From his arm by venepuncture
And cholesterol determined;
Why he had no clear conception
But there's wild enthusiasm
For cholesterol in serum;
As the children round the camp-fire
Dance and shout in exultation,
So there's wild enthusiasm
For cholesterol in serum:
Why it rises on infusion
Of suspended phospholipids
(Ethanolamine and choline
Joined to phosphatidic acid
With unsaturated acids—
Polyethenoic acids—
Also saturated acids,
Sticking out from off the sugar
Like the branches of a cactus),
Coming out from unknown tissues—
Red cells, liver, spleen and kidneys,
Atheromatous aortas,
Hepatectomized adrenals;
Why it falls, when you have eaten
Polyethenoic acids
Or essential fatty acids.

Having thus established clearly
What the normal value should be,
Hiawatha took a patient
Who had grave thrombotic symptoms,
Used his methods on the serum
(Methodology he called it),
Found a curious lipid in it—
Ante-iso-*trans*-oleic;
Recognized it by the usual
Gas-chromatographic method,
By the humps upon the paper
Like the Rockies at the sunset,
By the bumps upon the paper
Like the molehills in the prairies,
By a very curious spicule
Like the tower of Hotel Claremont
Coming in a new position
Which unquestionably proved it
Anti-iso-*trans*-oleic;
Called it Hiawatha's UFA,
"Hiawathianic acid";
No one else had found this lipid
In the serum of patient;
Called it Hiawatha's syndrome,
Hiawatha's lipidosis,
But he did not know his patient
Had been bitten by a viper—
Viperus Russellianus—
And in Russell's viper venom
There is but one type of UFA—
Ante-iso-*trans*-oleic,
Hiawathianic acid.

THERAPY

So he started quick to treat him;
Gave him safflower oil and corn oil,
Gave him pints and quarts of corn oil,
Gave it by infusion, also
Gave it by inunction, also
Poured it down, *per os*, his pharynx,
(As the beaver in the flood-time
Being drowned in swirling waters
Soon becomes a bloated carcass,)
Every orifice was needed
For administ'ring the doses
Of essential fatty acids;
But the patient still had in him
Ante-iso-*trans*-oleic,
Hiawathianic acid.

So he tried specific treatment;
Gave some linoleic acid
(Octadecadienoic),
Gave arachidonic acid
Named you might suppose from peanuts
But it is not found in peanuts
And is plentiful in spiders,
So perhaps he spelt it wrongly—
So "arach*n*idonic acid,"
Like arachnodea mater
Which as everyone remembers
Is the inmost spidery mother
Which ensheaths and wraps the cortex;
But the patient still had in him
Ante-iso-*trans*-oleic,
Hiawathianic acid,
Which had come if he had known it
From the Russell's viper venom.

SUMMARY

The moral of this story is then
Take some care when you have eaten
Butter, sardines, walrus liver,
Margarine or safflower seed oil,
Or arachidonic acid,
Or the body-fat of quokkas,
Or adrenals of the musk-rat,
Or the milk of female reindeer;
To avoid thrombosis don't get
Bitten by a Russell's viper
Which has but one type of UFA—
Ante-iso-*trans*-oleic,
Hiawathianic acid.

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Determination of Fatty Acids in Small Amounts of Plasma and in Lipid Components of Tissues by Ultraviolet Spectroscopy

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METHODS for determining fatty acid composition of fats and fatty materials, based on ultraviolet absorption spectroscopy of the alkali-isomerized fatty acids and iodine values, have been employed extensively for the past decade. The application of these methods for fatty acid analysis of small amounts of plasma or other tissues, however, has received less attention. In this discussion an attempt is made to show how these methods can be successfully applied to determine polyunsaturated fatty acids in one milliliter or less of plasma, and in small fractions obtained in separation of tissue lipid components.

(A) DETERMINATION OF POLYUNSATURATED ACIDS IN PLASMA

Solvents: The type and purity of solvents for extraction of lipids from plasma or other tissues are extremely important. In our studies, it was found necessary to carefully redistill each solvent through a 30-inch Vigreux column to insure that it was free of nonvolatile residues and low in spectral density. The following absorbances of a number of solvents were found satisfactory.

Absorbances (1 cm cell/distilled water)

	220 mμ	233 mμ	268 mμ
Methanol	0.210	0.070	0.00
Methylal	0.560	0.260	0.00
"Iso"-octane*	0.333	0.037	0.00
Ethanol	0.175	0.062	0.00
Ethyl ether	0.720	0.252	0.015

* 2,2,4-trimethyl pentane.

For 0.5 to 2 ml of plasma: If only small amounts of plasma are available, the fatty acid analysis is limited to determination of polyunsaturated acids, owing to the small amount of lipid fatty acids represented. In these cases the alkali-isomerization and subsequent spectral analysis are conducted on the extracted lipid without weighing the lipid and without prior saponification and removal of unsaponifiable matter, as follows:

The measured or weighed plasma (0.5 to 2.0 ml, or 0.5 to 2 g) was added dropwise to 15 ml of Delsal[1] solvent (methylal-methanol, 4:1) in a 50 ml centrifuge tube with mechanical stirring. After stirring for 30 min, the solids were separated by centrifugation, and the clear solution was transferred to another 50 ml centrifuge tube and taken to dryness in a water bath at 55° C in a stream of nitrogen. The solids from the first extraction were extracted again with 10 ml of the solvent, centrifuged as before, and the second extract added to the lipid residue from the first extraction and taken to dryness. The total lipid residues were then extracted with two 10 ml portions of isooctane, the extracts clarified by centrifugation and transferred to a 25 ml volumetric flask. The solution was diluted to 25 ml and thoroughly mixed. Absorbances (before isomerization) were then determined with isooctane as blank at 233, 268, 315, 346, and 374 mμ and absorp-

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tivities were calculated at these wavelengths, the concentration being expressed in milliliters or grams of plasma represented by 1,000 ml of the lipid solution.

The isooctane solution of lipid was transferred quantitatively from the absorption cell and the volumetric flask to a 50 ml round-bottom flask and evaporated under nitrogen on a water bath at 60° C to about 3–5 ml volume. This concentrate was then transferred quantitatively with a dropping pipette to an isomerization tube,[2] which was clamped in a water bath held at 60° C, and the solvent was removed under nitrogen. (At least three 2 ml washes were used to transfer the lipid, at no time allowing the solution in the isomerization tube to exceed about 5 ml). When the lipid residue appeared dry, 2 g* (+0.02 g) of 21 per cent KOH-glycol reagent[2] was added to the isomerization tube containing the residue and to another tube as a blank. The tubes were flushed with nitrogen prior to addition of reagent, and, immediately after, were connected to the nitrogen flow and placed in isomerization bath at 180° C for 1 minute and shaken for 5 seconds in the bath with a back and forth motion. The shaking operation was repeated twice after 30-sec intervals. The total heating time in the bath was 16 minutes, after which the tubes were removed with nitrogen flowing and cooled in a beaker of cold water. The contents of the tubes were transferred quantitatively to 25 ml volumetric flasks with methanol, diluted to the mark, and thoroughly mixed. The absorbances were measured in the analytic spectral regions in the usual manner,[2,3] and the absorptivities calculated as before, again referring the concentration to the milliliters or grams of plasma represented by 1,000 ml of the lipid solution.

The initial absorptivity (before isomerization) at each analytical wave length was subtracted from that obtained after isomerization to give corrected absorptivities (a'_c). The latter, multiplied by 1,000 to express results in terms of mg per 100 ml or per 100 g, were substituted in the following formulae for (a) in the calculation of polyunsaturated acids.

Plasma, mg/100 ml

Hexaenoic acid $= 3.41a_{374}$

Pentaenoic acid $= 1.449a_{346} - 1.353a_{374}$

Tetraenoic acid $= 1.650a_{315} - 1.667a_{346} - 0.079a_{374}$

Trienoic acid $= 1.105a_{269} - 0.879a_{315} + 0.190a_{346} - 1.251a_{374}$

Dienoic acid $= 1.092a_{233} - 0.573a_{269} = 0.259a_{315} - 0.033a_{346} - 0.260a_{374}$

Comparisons were made of analysis of polyunsaturated acids of plasma obtained by the above procedure ("micro") with those obtained from a large quantity ("macro") of the same plasma (after saponification of extracted lipid and removal of unsaponifiables). In the latter case the fatty acids were weighed and the isomerization conducted on weighed portions of fatty acids. A summary of the comparison is shown by the following data:

Acid	"Micro" *mg/100 ml*	"Macro" *mg/100 ml*
Dienoic	43.2	40.1
Trienoic	3.9	3.6
Tetraenoic	11.5	10.0
Pentaenoic	3.9	3.0
Hexaenoic	Trace	1.4

If larger amounts of plasma (>2 ml) are available, the lipids may be extracted in similar manner by using proportionately greater amounts of solvent. The extracted lipid can be saponified, and the fatty acids recovered quantitatively and weighed. The weighed fatty acids may then be made up to a convenient known volume in isooctane, and aliquots taken for iodine numbers and spectrophotometric analysis, thus permitting calculations of oleic and saturated acids[5] as well as of polyunsaturated acids.

(B) DETERMINATION OF FATTY ACIDS IN LIPID COMPONENTS OF TISSUES

Silicic acid chromatographic adsorption technics for separating tissue lipids into their principal components, i.e., cholesterol esters, glycerides, free sterols, and phospholipids, have been

* The isomerization procedure and apparatus are the same as described previously,[2] except for the amount of reagent and total time of isomerization. Repeated analysis on a wide selection of different fats and oils by the modification showed good agreement with those obtained by the method as published.[2] The constants and equations are also the same except those for hexaenoic acids which are based on values published by Hammond and Lundberg.[4]

in use for over six years. However, very little has been published concerning the fatty acid composition of the separated fractions, possibly owing to the general lack of experience with the application of the alkali-isomerization spectrophotometric method to small samples. Although it appears that gas-liquid partition chromatography may eventually be the method of choice in determining the fatty acid composition, considerably more research is required to make it a practicable quantitative technic for the various polyunsaturated acids.

Fractionation of Lipids on Silicic Acid Columns: Following the work of Borgstrom,[6] a number of modifications of silicic acid chromatography have been employed for the separation of lipids.[7,8,9] Some of the modifications probably came about as a result of lack of uniformity in the activity of different grades and lots of silicic acid. In general, however, the order of separation and nature of eluting solvents used were similar, certain changes being required to effect the separation desired with the particular grade of silicic acid on hand. The following procedure is a modification found satisfactory in our laboratory. The apparatus was similar in general design to that described previously[10] for fractionation of methyl esters, except that only glass joints were used and the dimensions of the glass column were different. It was equipped for operation under constant pressure of nitrogen which, however, could be varied from 0 to several inches of mercury positive pressure. The glass column (21 mm x 300 mm) was packed by introducing a slurry of 22 g of silicic acid* and filter aid† (80:20°) in 100 ml of redistilled petroleum ether (B.P. 35–60°). The slurry had been heated to boiling in a beaker with stirring for five minutes. During the addition of the slurry, slow stirring with a long thin stainless steel rod inserted in the column aided in uniformly packing the adsorbent as it settled by gravity. The adsorbent when settled in the column was about 190 mm in height. A receiver similar to that employed in collection of fractions under vacuum in distillations was used to collect measured volumes of eluate under nitrogen.

About 100 mg of weighed lipids in 10 ml of petroleum ether was added to the top of the column with an additional rinse of 10 ml of solvent. The first eluting solvent was then added to the separatory funnel, the space at top of column and the receiver flushed with nitrogen, and the flow rate adjusted to keep a constant level of liquid above the adsorbent. The drip rate through the column was adjusted to about 150 ml/hr by appropriate change of nitrogen pressure on the system. The order of elution and solvents employed were as follows:

(1) Hydrocarbons: 50 ml of petroleum ether + 1% ethyl ether.

(2) Sterol esters: 300 ml of petroleum ether + 1% ethyl ester.

(3) Glycerides: 300 ml of petroleum ether + 4% ethyl ester.

(4) Sterols + free acids: 350 ml of petroleum ether + 8% ethyl ester.

(5) Phospholipids: 250 ml of 3:1 ethyl ether-methanol followed by 250 ml of 1:1 ether-methanol.

In many instances, recovery of the material added to the column was substantially quantitative. Occasionally, some of the "phospholipid" components are not removed by this treatment and may require an additional treatment with chloroform-methanol 1:1. An alternate procedure in which the phospholipid fraction was first removed by precipitation with acetone and magnesium chloride solution (in ethanol) as described by Lipsky,[8] was found equally satisfactory. In this instance, the remainder of the lipid material in petroleum ether solution was put on the column, the order of separation of the other components being the same as before.

Treatment and Analysis of Eluted Fractions: The solvent was removed from the eluted fractions under reduced pressure of a water pump, and the lipid transferred quantitatively with small portions of petroleum ether to equilibrated and weighed (tared) 50 ml round bottom flasks. The solvent was then removed by

* Mallinckrodt's Analytical Grade (100 mesh), "Suitable for Chromatographic Analysis by the Method of Ramsey and Patterson."

† Hyflo Super-cel filter aid. (The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of similar nature not mentioned.)

evaporation under nitrogen, and finally under high vacuum and releasing to atmospheric pressure with oxygen-free nitrogen, until constant weight was obtained. The fractions were saponified with 0.5 N alcoholic KOH under nitrogen, the unsaponifiable material extracted with petroleum ether, and the fatty acids recovered by quantitative extraction after acidification with dilute sulfuric acid. The washed extract of fatty acids was taken to dryness and weighed as described for the eluted fractions; the fatty acids were then made up to standard volume with redistilled petroleum ether or isooctane. Aliquots were taken for determination of polyunsaturated acids by the alkali-isomerization spectrophotometric method as described for plasma lipids, except that in this case the weight of fatty acids is known and the concentration for calculating absorptivity is of course based on this weight.

The iodine values were determined on aliquots of the weighed fatty acids, representing 2 to 5 mg of the sample. The aliquots were added to 50 ml glass-stoppered flasks, the solvent removed under a stream of nitrogen, and 0.4 ml of chloroform added from a hypodermic syringe in such manner as to rinse the wall of the flask. Exactly 1.0 ml of 0.2 N Wijs solution was added with a micro-pipette, and the stoppered flasks were stored in a dark cabinet for 20 minutes. After this reaction time, the stoppers were loosened enough to permit washing by dropwise addition of 0.4 ml of 15 per cent KI solutiion followed by about 4 ml of distilled water. The liberated iodine was titrated with 0.01 N thiosulfate solution, 4 to 8 drops of 1 per cent starch solution being added as indicator.

The data in Tables I and II are presented primarily to serve as examples of the type of information obtainable by application of alkali-isomerization spectrophotometric analysis in conjunction with fractionating technics, such as chromatographic separation of lipids. Actually the spectrophotometric method should also prove to be useful adjunct in analysis of fractions obtained in gas chromatographic separations of methyl esters of fatty acids. Information of the type represented would appear to be valuable in connection with nutritional or biochemical investigations designed to show the effect of specific dietary fat constituents on lipid metabolism or in metabolic disorders.

TABLE I

Typical Separations of Component Lipids from Different Tissues

	A %	B %	C %	D %
Hydrocarbon	None	2.9	0.9	0.3
Sterol esters	28.0	40.3	19.9	1.2
Glycerides	19.4	14.1	9.9	13.4
Free sterols	38.9	8.3	3.0	3.2
Fatty acids	Trace	2.3	3.4	2.5
Phospholipids	13.3	32.2	62.4	78.2

A—Lipids from human atheromatous plaques.

B—Lipids from human plasma (healthy young men, post absorptive).

C—Lipids from rabbit plasma (adult males, post absorptive).

D—Lipids from rabbit liver (adult males, post absorptive).

TABLE II

Typical Analysis of Fatty Acids Obtained from Cholesterol Esters of Different Tissues

Acid	A %	B %	C %
Dienoic	23.6	47.4	49.5
Trienoic	1.4	0.7	1.7
Tetraenoic	7.0	8.0	3.0
Pentaenoic	1.3	1.0	0.4
Hexaenoic	1.0	0.7	None
Oleic	61.5	23.5	37.2
Saturated	4.2	18.7	8.2
Iodine values	134.0	142.7	139.3

A—Lipids from human atheromatous plaques.

B—Lipids from human plasma (healthy young men, post absorptive).

C—Lipids from rabbit plasma (adult males, post absorptive).

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Alkali-Isomerization Reactions of Unsaturated Fatty Acids

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ONE of the major problems in lipid chemistry and biochemistry has been the complete analysis of fatty acid composition of lipid materials. Prior to 1940, the fatty acid composition of fats and other lipid materials could be determined only in the most simple cases, and then only by rather tedious methods.

The composition of mixtures containing only saturated fatty acids and such common unsaturated fatty acids as oleic, linoleic, and linolenic could be ascertained with reasonable accuracy, but the quantitative analysis of more complex mixtures containing fatty acids of longer and shorter chain lengths, and fatty acids with 4, 5, and 6 double bonds, was quite impossible. Analysis of the fatty acid composition of the simpler fats and oils involved lead salt separations, and iodine value and thiocyanogen value determinations.

A more simple and useful method of fatty acid analysis became possible as a result of the development in the early 1940's of the ultraviolet spectrophotometer as a common laboratory instrument, and the discovery that unsaturated fatty acids, by suitable chemical treatment, could be made to absorb at certain characteristic wavelengths in the ultraviolet region. At first, this method could be applied only to relatively simple mixtures, but in recent years, it has been extended so that now, in some cases, it may be used for the analysis of certain mixtures containing fatty acids with as many as six double bonds.

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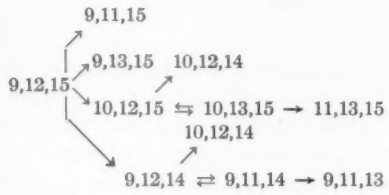
The common polyunsaturated fatty acids found in animal and vegetable tissues are characterized by methylene-interrupted unsaturation; that is, the double bond systems are separated by single methylene groups. In addition, in their natural state, most of the common polyunsaturated fatty acids have their double bonds almost entirely in the *cis* configuration. Structures of this type do not show any appreciable absorption in the near-ultraviolet range. However, it has been found that various reagents can shift the positions of protons along the carbon chain, a so-called prototropic shift, so that double bonds are moved into conjugated positions.

In the methods of analysis most commonly used at present, this isomerization is accomplished by means of strong alkali. The fatty acids to be isomerized are heated, at 180° C for a given period, with potassium hydroxide in ethylene glycol or glycerol, after which the isomerized mixture is diluted appropriately with solvents and its absorption in the ultraviolet region is determined.

When linoleic acid (two double bonds) is alkali-isomerized, one or the other of the double bonds tends to move predominantly toward the remaining double bond. The reason for this is that the conjugated diene thus produced is a relatively stable structure, thermodynamically. Thus, a mixture of 9,11- and 10,12-octadecadienoates is produced from normal linoleic acid. In addition, because the *trans* configuration is more stable than the *cis*, those double bonds that have moved are found predominantly in the *trans* configuration.

The conjugated dienes formed from linoleate by alkali-isomerization absorb ultraviolet light strongly in the region of 233 mμ. Conjugated trienes, however, absorb strongly at 268 mμ, and conjugated tetraenes at 315 mμ.

Normal linolenic acid is *cis, cis, cis*-9,12,15-octadecatrienoic acid. When this acid is alkali-isomerized, many isomers can be formed. The possible transitions are illustrated in the following diagram, and, under the conditions usually employed, the final product consists mainly of a mixture of all of these forms.



Thus, under normal conditions, the alkali-isomerization of linolenic acid results in the production of a mixture of conjugated dienes and trienes. Consequently, the product absorbs strongly at both 233 and 268 mμ. Similarly, the alkali-isomerization of arachidonic acid leads to a mixture of conjugated dienes, trienes, and tetraenes, absorbing at 233, 268, and 315 mμ, respectively.

The alkali-isomerization-spectrophotometric method of analysis is an empirical procedure, and it is necessary to establish spectral constants for the various pure polyunsaturated fatty acids by alkali-isomerizing under the conditions that subsequently will be employed in analysis. Measurement of ultraviolet absorption at the various wavelengths then establishes constants which may be employed in calculating the original composition of alkali-isomerized mixtures of polyunsaturated fatty acids.

A Note on Increasing the Accuracy of the Micro Alkaline Isomerization Technic for Determination of Unsaturated Fatty Acids

GEORGE D. MICHAELS, PH.D.*

THE alkaline isomerization technic has been used successfully for the analysis of vegetable oils. This method has been made more sensitive by increasing the concentration of the KOH used for isomerization[1] and has been used for determinations of small quantities of unsaturated fatty acids.

Two inherent difficulties of the original method in its adaptation for micro analysis were: *first*, the lack of sensitivity, especially in the area for tetraenoic acids, and, *second*, the high absorption in the ultraviolet by the reagent, especially at 233 mμ wavelength The first problem was satisfactorily solved by the increased KOH concentration, but the second problem was further complicated, since the alkali increased the absorption by the reagent.

Usually in macro analysis 100 mg of fatty acids are isomerized with 5 ml of the reagent. In micro analysis, 1 mg of fatty acids is usually isomerized with 1 ml of the reagent, a twenty fold difference in the proportion of reagent to fatty acids. In some micro-analyses of fatty acids the absorption by the fat may be less than 10 per cent of the total absorption!

The following simple procedure has been introduced to reduce the absorption due to the reagent blank. After cooling the isomeriza-

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tion tubes following the isomerization, about 2 ml water is added and sufficient hydrochloric acid to make the solution acid. Petroleum ether is then added, the tubes are shaken and the petroleum ether fraction is transferred. This is repeated several times to assure quantitative transfer of the fatty acids. The petroleum ether fraction is then evaporated to dryness under partial vacuum and the fatty acids are dissolved in sufficient methanol for the spectrophotometric reading.

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The Separation of the Long Chain Fatty Acids by Gas-Liquid Chromatography

A. T. JAMES, PH.D.*

ALL chromatographic systems consist of two phases: one phase is held stationary, the other moves through it. The stationary phase may be a solid or a liquid; in the latter case it is distributed over the surface of a finely divided microporous inert (i.e., nonabsorptive) solid, usually Celite or paper. The support and stationary phase are usually packed into a column. The moving phase can be either liquid or gas; thus we speak of a liquid-solid, a liquid-liquid, a gas-solid, or a gas-liquid chromatogram. Any substance added to a two phase system, when equilibrium is reached, will be found to have distributed itself between the two phases. The ratio of concentrations in the two phases is known as the distribution coefficient (α), and is a constant for the substance distributed, the two phases and the temperature. Any mixture of substances can be separated on a chromatogram provided all the components have different distribution coefficients.

Let us consider two substances A and B. Substance A at equilibrium has 90 per cent in the stationary phase and 10 per cent in the moving phase. Substance B however, has only 10 per cent in the stationary phase and 90 per cent in the moving phase. If a mixture of A and B is applied to the chromatogram and washed through with solvent, then B will move as a zone at nine tenths of the rate of the moving solvent (i.e., $R_F = 0.9$) and A will move as a zone at one tenth of the rate of the moving solvent (i.e., $R_F = 0.1$). Clearly substance B will move rapidly away from A and can be eluted from the chromatogram quite separate from substance A.

In the gas-liquid chromatogram (the latest type) the substances to be separated are moved through the column by a stream of permanent gas instead of by a solvent. This use of a gas as moving phase confers a number of advantages, viz:

(1) The compressibility of the gas allows the use of long thin easily packed columns that give high rates of flow by the application of quite small pressures.

(2) High diffusion rates in the gas allow high column efficiencies to be attained even when separations are carried out rapidly.

(3) It is easier to detect the zones, which emerge as vapors in the gas stream, than substances in solution and the detectors are easily made self recording, so that after loading the column the whole process is automatic.

The experimental arrangement of the gas-liquid chromatogram is very simple. The column itself consists of a length of glass or metal tubing packed with a mixture of a microporous support (Celite or crushed firebrick) of known particle size (usually 80–100 or 100–120 mesh) and the liquid phase—a substance liquid at the column temperature but having a very low vapor pressure (10^{-2} to 10^{-3} mm). The column is maintained at a constant temperature by a heating jacket and is connected to the vapor detector. The latter records automatically against time the concentration of vapor in the zone blown out of the column by the stream of gas at a constant flow rate. Despite this basic simplicity it is surprising how complicated an apparatus many workers have constructed.

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Gas-liquid chromatography has proved to be the most refined and rapid method for the quantitative and qualitative analysis of the long chain fatty acids.[1,2] In a great many problems it is necessary to deal with the smallest possible sample, e.g., the determination of fatty acid composition of repeated blood samples. The size of sample necessary is determined entirely by the sensitivity of the detector employed and unfortunately many detectors fall in sensitivity when the temperature of the apparatus is raised to the levels necessary for analysis of the long chain acids (170° → 250° C).

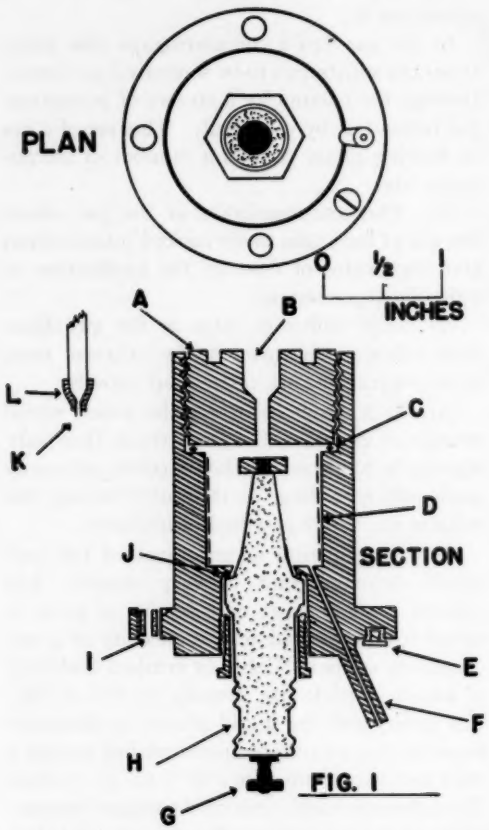

Fig. 1. Plan and section of argon β-ray ionization monitor. A. removable cap for insertion of Sr^{90} foil; B. column seating; C. copper gasket for top cap; D. Sr^{90} foil (Silver); E. screw contact for H.T. input; F. gas outlet; G. input contact; H. center section of demountable spark plug; I. ceramic insulator for mounting screws; J. copper gasket for center electrode; K. column base; L. silicone rubber sleeve to provide gas tight seal in column seat.

Recently a new detector has been developed by Lovelock[3] that has the merits of very high sensitivity even at elevated temperatures and also great simplicity. Its construction has recently been still further simplified.[4] The detector consists of a small chamber containing a cylindrical radioactive source (Sr^{90}, 10 millicuries) emitting β-particles. In the center of the chamber is placed an insulated electrode consisting of the center section of a demountable automobile spark plug fitted with a small disc electrode (Fig. 1). A high d.c. potential (300 to 1,200 volts) is maintained across the cell giving an ionization current of 10^{-8} amperes when pure argon (a commercial product) is used as mobile phase of the chromatogram. As soon as a zone passes into the chamber a large signal is generated that is amplified by a simple electronic circuit and fed to a recorder. The response is linear and independent of molecular weight for fatty acid methyl esters of molecular weight greater than 100. The detector is insensitive to changes in gas flow rate and relatively insensitive to temperature variations so that highly constant thermostatic control is unnecessary.

The column heater consists of a cylindrical bar of aluminium $2^1/_2$-inch diameter and 4 ft 3 in. long. A slot $^1/_2$ inch wide is machined along almost the whole length of the bar (Fig. 2) and partially closed by insertion of a length of $^1/_2$ inch section aluminium, leaving a square section hole to contain the column (4 ft packed length, 4 mm internal diameter). A cylindrical space is turned in the bottom of the bar to accommodate the detector cell and a hole is bored through to break into the slot. The detector is insulated electrically from the bar by a gasket of silica loaded silicone rubber and held in place by screws passing through ceramic insulators. The bar is heated by a double winding of glass insulated Nichrome wire (a total dissipation of 150 watts will give temperatures up to 250° C), a resistance thermometer being used to control the switching of one of the windings, the input to both windings is controlled by a variable transformer. The apparatus has proved cheap to construct, robust and

TABLE I

Comparison of Sensitivity of Various Detectors

Type of detector	Approximate useful sensitivity achieved in terms of concentration of vapor detectable at 200° C	Approximate loads of fatty acid esters necessary for analyses of fatty acids in the range C_8–C_{22}
Katharometer	1 in 10^5	3 to 20 mg
Gas density meter[5]	1 in 5×10^5	1 to 3 mg
Hydrogen flare detector[6]	1 in 10^9	100 μg to 1 mg
Argon β-ray detector[3]	1 in 10^{12}	10 to 100 μg

highly sensitive. In Table I is given a comparison of the sensitivity of various detectors. The quoted load for the argon detector is larger than necessary at maximal sensitivity but it is difficult to load a column accurately with quantities of less than 50 μg. A typical record is shown in Figure 6.

FACTORS INFLUENCING THE SEPARATION OF THE LONG CHAIN SATURATED AND UNSATURATED FATTY ACIDS

The relative position of the zones on a gas-liquid chromatogram is controlled by the differences in partial vapor pressure of the substances when dissolved in the column stationary phase. The nature and magnitude of the cohesive forces between solute and solvent molecules defines the partial vapor pressure of the solute.

When the stationary phase of the chromatogram is a saturated paraffin hydrocarbon, the cohesive forces between the solvent and solute molecules are solely of the nonpolar Van der Waals type. These forces decrease with decrease in molecular weight, hence an unsaturated compound will be held less strongly than the corresponding saturated compound. In Figure 3 is shown a plot of log time of emergence of the center of the zone (relative to a master substance, *n*-pentane) against number of carbon atoms in the molecule for a variety of unsaturated hydrocarbons. In this case the stationary phase is a saturated paraffin hydrocarbon. The dotted line represents the same data for the corresponding straight chain saturated compounds. It can be seen that the unsaturated straight chain hydrocarbons move more rapidly through the column than the corresponding saturated compounds. The same is true for the long chain fatty acid methyl esters. In Figure 4 is shown a separation of these acids using as stationary phase Apiezon L grease. Both linoleic and oleic acids move ahead of stearic acid though linoleic and linolenic acids are not resolved. However, this type of column possesses the advantage that zone position is affected not only by number of double bonds but also by their position and configuration (i.e., whether *cis* or *trans*). The peak in Figure 4 following closely on oleic acid is due to positional isomers of oleic acid.

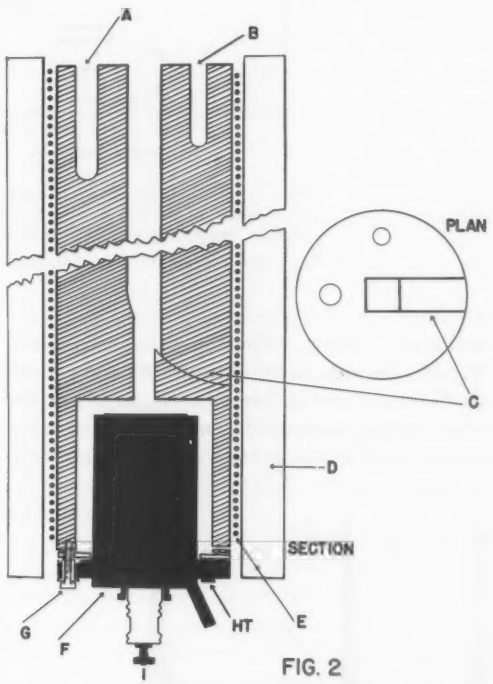

Fig. 2. Plan and section of heating jacket. A. pocket for resistance thermometer; B. pocket for thermometer; C. inserted section to form space for column; D. fiberglass pipe insulation; E. heater winding; F. detector cell; G. holding screws passing through ceramic insulator.

When a more polar liquid is used as the stationary phase new types of solute-solvent interaction come into play. In addition to Van der Waals forces, polar groups such as double bonds show a selective interaction with the

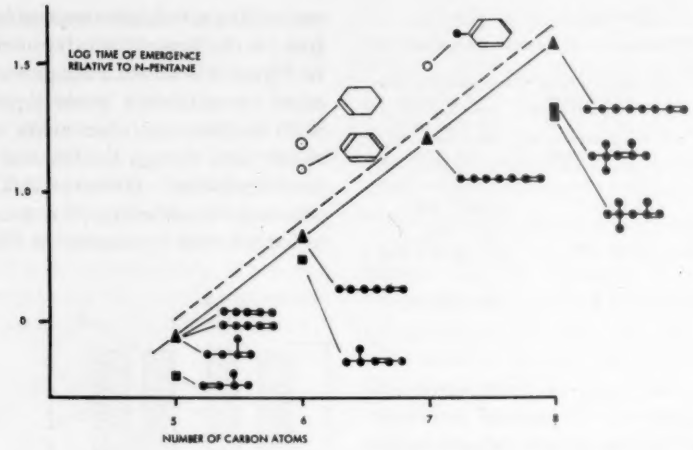

Fig. 3. Relationship between log-time of emergence (relative to *n*-pentane) and number of carbon atoms in the molecule of some unsaturated hydrocarbons. The dotted line refers to the straight chain saturated hydrocarbons. Each dot represents a carbon atom. Stationary phase—liquid paraffin at 78°.

stationary phase. This is demonstrated in Figure 5 for the same unsaturated compounds as Figure 4 but in this case the liquid in the column is an aromatic hydrocarbon. Now the unsaturated hydrocarbons are held back relatively to the corresponding saturated hydrocarbons. Similar effects will occur in polar liquids with long chain fatty acids. The first demonstration of these effects was given by Callen and Orr[7] using a polymer of propylene

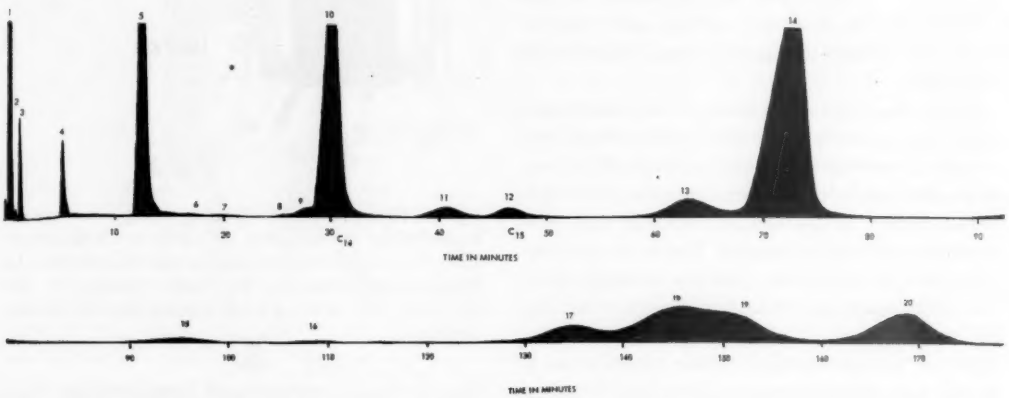

Fig. 4. Separation of 3.6 mg of fatty acid esters from human fecal fat. Stationary phase—Apiezon L. vacuum stopcock grease. Temperature 197° C. Column length 4 ft, nitrogen pressure-inlet 65 cm above atmospheric, outlet at atmospheric pressure. Detector—gas density meter.

Peaks in order of appearance: (1) Arr.; (2) *n*-hexanoic; (3) *n*-octanoic; (4) *n*-decanoic; (5) *n*-dodecanoic; (6) branched decanoic; (7) *n*-tridecanoic; (8) highly branched tetradecanoic; (9) branched saturated + mono unsaturated tetradecanoic; (10) *n*-tetradecanoic; (11) *iso* + ante *iso* branched pentadecanoic; (12) *n*-pentadecanoic; (13) branched saturated + mono unsaturated hexadecanoic; (14) *n*-hexadecanoic; (15) *iso* + ante *iso* branched heptadecanoic; (16) *n*-heptadecanoic; (17) linoleic; (18) oleic; (19) positional isomers of oleic largely vaccenic acid; (20) *n*-octadecanoic.

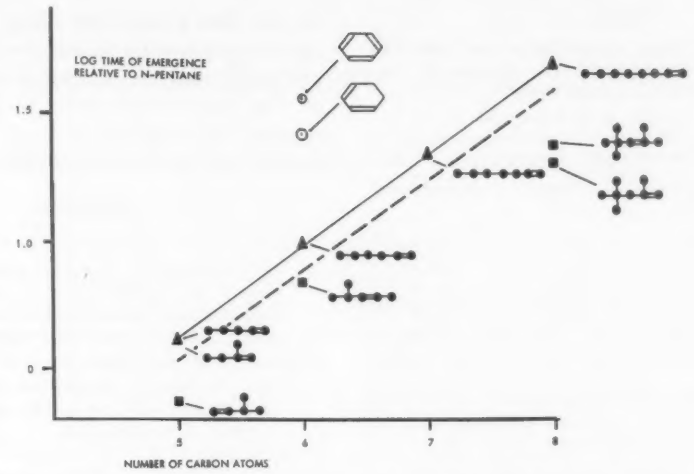

Fig. 5. Relationship between log-time of emergence relative to *n*-pentane and number of carbon atoms in the molecule for some unsaturated hydrocarbons. Stationary phase—benzyldiphenyl at 78°.

glycol and adipic or sebacic acids (Reoplex 400, Geigy Chemical Co. Ltd.). In my hands, however, such polymers have proved thermally unstable and have been stripped rapidly from the column at 180–200° C. I have preferred to use the easily synthesized polymers of ethylene glycol and adipic acid since these are thermally stable and give excellent separations of all the common saturated and unsaturated acids. Double bond position does not, however, alter chromatographic behavior. Lipsky and Landowne[8] have reported the use of polymers of diethylene glycol and adipic acid and have shown what refined separations are now possible. In Figure 6 is given an example of the analysis of only 100 μg of mixed fatty acids on a 4 ft column with a stationary phase of polyethyleneglycol-adipate. The column efficiency is 3,500 theoretical plates. All the polar stationary phases mentioned possess the advantage that the retention volumes of the acids (i.e., time of emergence × flow rate of carrier gas) are lower than with the saturated hydrocarbon stationary phases, so that analyses can be carried out more rapidly even at lower temperatures.

The only other micro method for the analysis of the polyunsaturated acids, the alkali isomerization technic, is rather time consuming. A comparison has been made of the results obtained on similar samples by this technic and with the gas-liquid chromatogram. The comparison of analysis for a wide range of linoleic and linolenic acids is given in Table II (a), and for arachidonic acid in Table II (b). Good agreement is obtained suggesting that

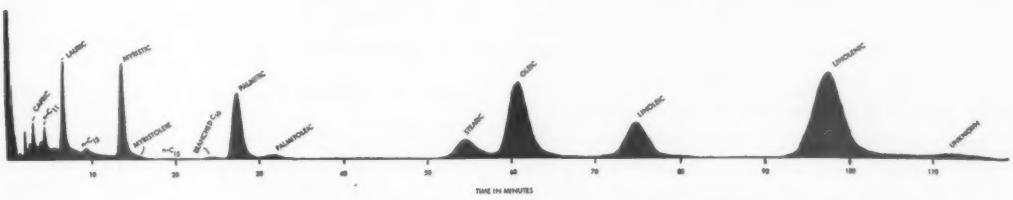

Fig. 6. Separation of 100 μg of fatty acid methyl esters. Stationary phase polyethylene glycol adipate. Temperature 175° C. Column length 4 ft. Argon pressure at inlet 65 cm above atmospheric; at outlet—atmospheric pressure. Detector—Argon β-ray monitor.

TABLE II

Comparison of Alkali Isomerization and the Gas-Liquid Chromatogram in the Analyses of Polyunsaturated Acids

(a) Linoleic + linolenic acids

By alkali isomerization			By gas chromatogram
Linoleic %	Linolenic %	Total %	%
48.1	4.8	52.9	59.5
44.0	7.0	51.0	52.5
30.4	5.2	35.6	33.6
14.0	7.8	21.8	21.9
2.6	1.25	3.85	4.6

(b) Arachidonic acid

% Arachidonic	
By alkali isomerization	By gas chromatogram
0	0
0.3	0.5
4.9	4.5
1.5	1.33
22.9	22.8

the gas chromatogram is capable of providing accurate analyses for these acids as well as for the saturated acids. It should, however, be mentioned that the lack of even impure samples of the long chain penta- and hexaenoic acids has prevented any study of their behavior on the chromatogram; this remains to be done.

To summarize the present position, it can be said that a relatively cheap and simple apparatus can now be constructed that will allow automatic analyses of microgramme quantities of long chain fatty acids to be carried out rapidly and accurately so that studies of lipid metabolism will be greatly facilitated.

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Lipids of Biologic Importance

Peroxidation Products and Inclusion Compounds of Lipids

W. O. LUNDBERG, PH.D.*

THIS paper is devoted to a brief consideration of two special classes of lipid materials which may not have received the attention they deserve in connection with metabolic problems in the lipid field.

One class consists of the various autoxidation or peroxidation products of lipid materials, particularly the initial peroxidation products of fatty acid esters. The second class consists of so-called lipid inclusion compounds, the existence of which has been recognized vaguely for many years but about which little definite information has been available until recently.

These two classes of materials are not related in any direct sense. From a biologic standpoint, peroxidation products usually are regarded as abnormal lipids. The inclusion compounds, on the other hand, are regarded as biologically normal substances, but their biologic importance has been little recognized. In another sense, however, there is a relationship between these two classes, in that the existence of the second prevents formation of compounds of the first. This will become more apparent as the discussion progresses.

PEROXIDATION PRODUCTS OF NORMAL LIPIDS

(A) Nature of Peroxidation Reactions

The products of these reactions are formed by the interaction of free or loosely bound molecular oxygen with lipid materials, at or in the vicinity of unsaturated centers. Most unsaturated fatty acid esters react directly with oxygen of the atmosphere, initially forming peroxides, most of which are hydroperoxides; hence the term "peroxidation." One of the best known features of the *in vitro* reaction is its autocatalytic nature; hence the term "autoxidation." It has been established that the autoxidation reaction is a free radical chain reaction, and that the hydroperoxides formed in the initial phases are themselves the catalysts that are responsible for acceleration of the oxidation as the reaction progresses.

In general, the oxidizability or the rate of autoxidation increases with increasing unsaturation, and esters of polyunsaturated fatty acids, such as arachidonic or hexaenoic acid, oxidize many times more rapidly than esters of oleic acid under the same conditions.

The rate of oxidation also is accelerated by various other catalytic influences. Thus, various forms of light energy, such as visible light, ultraviolet light, x-rays, and nuclear radiations accelerate autoxidation. Light photons of low energy do this by accelerating decomposition of peroxides to give chain-initiating free radicals; with radiation of higher energies, the production of free radicals from unoxidized lipid molecules also is involved.

The oxidation of polyunsaturated fatty acid compounds that contain *cis, cis*-1,4-pentadiene groups is accelerated by lipoxidases. These enzymes are found in various leguminous plants, but as yet their presence in animal tissue is problematical. The enyzmes catalyze the production of hydroperoxides very similar to those formed in ordinary autoxidation, except that the peroxides are optically active.

The formation of lipid hydroperoxides is catalyzed also by various metallic ions, in some

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cases at very low concentrations. Copper or iron at a concentration of 1 p.p.m., or less, markedly accelerates the autoxidation of lipid materials in general. Certain iron-containing biologic substances are very effective catalysts, particularly hematin compounds such as cytochrome C and hemoglobin.

Because of the chain nature of the autoxidation reaction, it also may be inhibited by very small amounts of certain substances. Among biologic substances, the various forms of vitamin E, i.e., the tocopherols, are the best known inhibitors of lipid oxidations. Vitamin E is found in small amounts in almost all natural fats and oils. In general, various polyphenolic substances are effective as antioxidants or inhibitors of autoxidation because of their ability to interrupt the propagation of autoxidation reaction chains. However, vitamin E is considered to be a biologic antioxidant, also, and, largely on the basis of circumstantial evidence, may conceivably be involved in preventing *in vivo* oxidations by some other mechanisms.

The autoxidation of lipid materials does not end with the formation of peroxides. As already indicated, peroxides or hydroperoxides decompose, and this decomposition is more rapid with hydroperoxides of more unsaturated lipids. At the same time, further oxidation, and, also, polymerization, may take place. Thus, as oxidation progresses a host of secondary oxidation products is formed from fatty acid esters, including various aldehydes, ketones, acids, and other substances.

{B} Biologic Importance of Autoxidized Lipids

Autoxidized or peroxidized lipid materials are important nutritionally and in other biologic ways. The autoxidation of fats is responsible for rancidity in fat-containing food products. The prevalence of oxidative rancidity in foods is far greater than is suspected generally. Frequently, the rancid flavor and odor of oxidized fats are hidden by other flavor components of food products so that, although the over-all flavor of a product may be regarded as inferior, the presence of fat rancidity is not recognized.

Until recent years, rancidity was considered objectionable primarily from the standpoint of aesthetics. Now, however, it is recognized that rancid fats in food products are objectionable because of nutritionally harmful qualities, although the extent of their harmfulness has not yet been assessed adequately. It is known that oxidized fat contributes to a rapid destruction of autoxidizable vitamins, such as the fat-soluble vitamins A, D, and E, and that it contributes to the destruction of various other nutrients, also. There are increasing evidences, also, that fat peroxides and other autoxidation products may have certain direct toxic effects.

Another area in which lipid peroxidation products may be important concerns the effects of high-energy radiation on tissue. It has been found that, when animal tissues are irradiated with high-energy radiations, peroxides are formed, including peroxides of lipid materials. These peroxides have been implicated in carcinogenic and other toxicologic effects of high-energy radiations. However, much remains to be done to elucidate the role of the lipid peroxides in this field.

It has been shown that, with some animals, there is an accumulation of fat peroxides and other lipid oxidation products in vitamin E deficiency. The peroxides accumulate in adipose tissues, as well as in the fatty components of various other tissues. In vitamin E-deficient rats, brown pigment forms in muscle tissue, which is believed to be a high polymer formed from oxidized fat.

It has been reported, also, that peroxides are present in the lipids of atheromatous plaques. Their role and importance in relation to atherosclerosis and heart disease, however, remain to be determined.

In summary, it may be stated that products formed by the reaction of free or loosely bound oxygen with lipid materials do have importance in biologic systems, although, in general, they are to be regarded as abnormal biologic substances.

LIPID INCLUSION COMPOUNDS

It long has been known that soybeans and various other oil-containing seeds may be stored for relatively long periods without any demonstrable oxidation of their lipid content.

This cannot be explained satisfactorily on the basis of impermeability of the seeds to oxygen.

Again, although blood contains hemoglobin and oxyhemoglobin, which are powerful agents for promoting lipid oxidations *in vitro*, normally there appears to be no appreciable peroxidation of any of the lipid materials in blood.

Vitamin E is known to be present to an appreciable extent in soybeans and, to a lesser extent, in normal blood. Nevertheless, the amounts present are quite insufficient to account for the inhibition of lipid oxidation, at least on the basis of any antioxidant mechanism that has been observed *in vitro*. These observations provide a point of departure for consideration of another unusual class of lipid materials, namely, lipid inclusion compounds.

Although, for many years, complexes of lipids with various materials had been known to exist, it was not until 1941 that a clear insight into the nature of some of the simpler inclusion compounds was obtained. It was discovered that straight chain aliphatic substances, such as fatty acids and their esters, would form crystalline complexes with urea, in which molecules of urea were arranged in a spiral structure around the aliphatic carbon chain. The urea was regarded as a "host" substance, and the straight chain lipid material, a "guest" substance.

It has since been discovered that various other relatively simple molecules can serve as host molecules for lipid materials, including thiourea, desoxycholic acid, and Schardinger dextrins. In more complex inclusion compounds, molecules of high molecular weight may serve as host molecules, and the inclusion compounds themselves, although having highly organized structures, are frequently non-crystalline.

At ordinary room temperatures, linoleic acid is a colorless liquid. However, it readily forms an inclusion compound with urea, in the form of a crystalline solid. One of the most remarkable features of this complex, and a feature of lipid inclusion compounds in general, is that it is completely stable to autoxidation. The urea-linoleic acid complex may be exposed to the atmosphere for many months without showing any measurable uptake of oxygen.

Linoleic acid has come to be known as one of the "essential" fatty acids. In purified form, it is also quite susceptible to oxidation. In order to eliminate the problem of the destruction of linoleic acid by autoxidation in experimental animal diets, it now is the practice, in some laboratories, to add linoleic acid to the diet, in the form of its urea complex. The essential fatty acid activity of the complex is comparable to that of an equivalent quantity of pure linoleic acid. The complex evidently is broken down completely in the gastrointestinal tract.

More recently, it has been found possible to stabilize vitamin A as an inclusion complex in cornstarch. A variety of other lipid inclusion compounds have been observed and characterized in the past several years.

It is highly probable that these lipid inclusion compounds are of great importance in nature, and, that, in many instances, the existence of inclusion compounds is responsible for the stability of those lipid materials which, in their isolated state, are highly unstable to atmospheric oxygen.

Column Chromatographic Fractionation of Plasma Lipids

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PARTIAL dehydration of silicic acid will increase its adsorptive property for fats to a maximum. Further dehydration will decrease the adsorptive properties. The anhydrous acid has practically no adsorptive properties.[1]

The degree of hydration not only affects the amount of fats which each gram of the acid may adsorb, but also the type of solvent needed for their elution. A specific example is that of cholesterol esters; when added to a column of silicic acid containing from 20 to 22 per cent moisture, the esters may be quantitatively eluted with straight petroleum ether. However, cholesterol esters added to a column of silicic acid containing 15 per cent or less water of hydration cannot be eluted with petroleum ether alone but must be eluted with a mixture of petroleum ether and diethyl ether.

Since there are no simple methods for controlling the degree of activation, we have found that the most reliable and reproducible results may be obtained by using silicic acid as it is generally obtained from the manufacturer. This contains 20 to 22 per cent moisture. It is well to specify that the acid on ignition shall have a loss of from 20 to 22 per cent by weight.

The columns used consist of 25 ml burettes with self-lubricating teflon plug assemblies* and 125 ml flasks fused to the top of the burettes as reservoirs. A glass wool plug is introduced, and a mixture of equal parts by weight of silicic acid and celite is added, with tamping, to a height of 10 cm. Plasma lipids dissolved in small amounts of petroleum ether containing not more than 30 mg may be added to this column. The tube containing the lipids is washed three times with 2 ml portions of petroleum ether which are added to the column and allowed to flow in without pressure until the liquid has become completely absorbed.

CHOLESTEROL ESTERS

Cholesterol esters are eluted with a total volume of 100 ml petroleum ether under slight positive pressure to maintain a flow rate of approximately 1 ml/minute. The efficiency of the separation is checked by comparing the values for free and total cholesterol determined on the eluate and those obtained by direct analysis of the original plasma lipid filtrate and with values for total fat determined by Bragdon's method on the eluate.[2] In a series of 29 eluates the values differed from the direct determination of esterified cholesterol on the same filtrates by an average of 7.3 ± 5.1 mg % which was 4.8 ± 3.7 per cent of the direct values.† No free cholesterol was present in the eluates nor were other lipid fractions eluted with the cholesterol esters. This last fact was confirmed by the good agreement shown between values for the total fat determined on

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* Ultramax-Fischer, and Porter Co.

† Standard error of the mean.

the aliquots of the eluates and the total fat values calculated from the ester cholesterol using the factors given by Bragdon for cholesterol esterified with a C_{18} fatty acid. In the same series, the mean difference between total fat determined directly and calculated was 9.00 ± 3.00 mg % or 6.2 ± 1.12 per cent of the direct value.

SEPARATION OF FREE CHOLESTEROL, TRI-, DI-, AND MONOGLYCERIDES

Free cholesterol and purified mono-, di-, and triglycerides were added separately and also as mixtures to the columns and different mixtures of petroleum ether and diethyl ether were used to determine the optimum mixture for their separation. If not more than 30 mg of glycerides are added to the column, 50 ml of 3 per cent diethyl ether in petroleum ether will quantitatively remove all the free cholesterol and triglycerides, but none of the mono- or the diglycerides. Ten per cent diethyl ether will elute the diglycerides. One hundred per cent diethyl ether will elute the monoglycerides quantitatively. Excellent recoveries were obtained when a mixture containing equal amounts of each of the glycerides was added to the column, and eluted successively as noted above. When mixtures of the glycerides were added to plasma lipid filtrates they were quantitatively recovered by appropriate elution.

In one such experiment pooled filtrates equivalent to 2 ml of plasma were run on duplicate columns using the solvents specified above. To check recovery of added glycerides, three other pairs of columns were run with 5 mg of either tripalmitin, dipalmitin, or monopalmitin added to the filtrate before evaporation. A fifth pair of columns was run with 5 mg of each added. Results of this experiment are shown in Table I. Variations in recovery are within the error of the method used to determine total fat.[2]

TABLE I

Recovery of Glycerides Added to Pooled Acetone-Alcohol Filtrate of Plasma

Results are expressed as mg of total lipid in the pooled eluate from two columns, equivalent to 4 ml plasma

No. of filtrate:	PF-I	PF-II	PF-III	PF-IV	PF-V
Substance added:	None	Tripal-mitin	Dipal-mitin	Mono-palmitin	Tri-, di-, and mono-palmitin
Petroleum ether	11.15	11.15	11.05	11.85	11.55
3% Diethyl ether in PE	9.90	18.65	11.05	9.65	20.40
10% Diethyl ether in PE	0.50	1.15	8.65	0.50	10.00
100% Diethyl ether	0	0.65	0.50	9.40	10.00

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Fat Absorption

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THE STUDY of absorption includes the reactions following digestion in the intestinal lumen and prior to appearance of the products in the blood or lymph. Since, in general, these reactions have not been well studied, the actual processes occurring during absorption are largely a matter of conjecture.

In the case of triglyceride absorption, some knowledge can be gleaned from the state of the triglycerides in the intestinal lumen and in the lymph or blood. Bollman, Cain and Grindlay[1] and others, using their technic for thoracic duct cannulation, demonstrated that most of the products of fat digestion appear in the lymph. Chaikoff and his collaborators,[2,3,4] however, found that in whatever form fed, the longer chain fatty acids appeared almost exclusively in the lymph, while those with less than 12 carbon atoms appeared to a greater extent in the portal vein.[5] The transition point appeared to be in the fatty acids with chain lengths of about 8–12 carbons, having a partition coefficient between water and fat of about unity (Table I).

Since most of the fatty acids of the diet are of the 16 to 18-carbon chain variety, it can be expected that the products of fat digestion will appear largely in the lymph. The exact form in which triglycerides are absorbed is in some doubt because of the possibility that following hydrolysis to the monoglyceride state, some resynthesis occurs.[6] At present it appears that glycerides of all sorts and fatty acids are probably absorbed as finely emulsified droplets of less than 0.5 μ in diameter.[7]

The reactions occurring in the intestinal cells lead to the formation of triglycerides and small amounts of phospholipids. The mechanisms of such reactions have been elucidated, largely by the work of Kennedy and his collaborators, and it seems logical to suppose that the same mechanisms occur in the intestinal cells as in cells of other types. It should be mentioned here that the starting materials in this case are probably the absorbed mono- or diglycerides and that the proportion of these two partial glycerides absorbed may determine the proportion of triglyceride and phospholipid formed in the intestinal cells. Triglyceride could be formed from diglyceride by reaction with acyl coenzyme A while monoglyceride could give rise to glycerophospholipid by reaction with ATP to form acyl glycerophosphate followed by reaction with unsaturated acyl coenzyme A to get the unsaturated acid in the α position. The resulting phosphatidic acid could then be transformed into phospholipids by the usual reactions. Triglycerides, which are also absorbed, could pass unchanged into the lymph.

The exact mechanism by which absorption occurs is not known. Frazer's hypothesis that small canals of about 0.5 μ diameter in the intestinal cells furnished the passage way[8] has been largely disproven.[9] At present it appears that finely divided lipid particles, emulsified with the aid of bile and hydrolysis products, may pass through the cell wall fairly readily in either direction, as will be seen later in discussions of fat transport.

TABLE I
Fatty Acids Transported by Thoracic Duct

Chain length	Per cent
18	95
16	97
14	70
12	35
10	10

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Little is known about the absorption of phospholipids. Although Artom and Swanson[10] demonstrated that some portion of the molecule could be absorbed unhydrolyzed, Bloom and co-workers[11] found that only about 17 per cent appears in the lymph as phospholipid. The remainder is apparently hydrolyzed to such an extent that the products are indistinguishable from those of triglyceride hydrolysis. It can be assumed that once both fatty acid residues are removed, the remainder of the glycerophosphatides will be absorbed into the portal system.

Since there is good evidence that the sterol esters are largely hydrolyzed in the small intestine[12] only the absorption of the sterols themselves need be considered.

Cholesterol absorption is strongly influenced by the presence of other substances in the small intestine. It has been reported many times that cholesterol fed in the absence of fat is not too well absorbed. Different types of fat, however, appear to have quite different actions. The greatest absorption, as measured by fecal cholesterol, occurs with concomitant feeding of oleic acid,[13] followed by the more unsaturated fats, as corn oil. Saturated triglycerides actually decreased cholesterol absorption. It has also been reported, however, that even with a cholesterol-free diet, the serum cholesterol levels of rats are higher with the more highly unsaturated fats.[14] Thus, fat is thought to have several effects on cholesterol absorption—stimulation of bile flow,[14] provision of fatty acid (especially polyunsaturated) for esterification, and some additional unknown influence. Mineral oil, as would be expected, decreases cholesterol absorption.[15]

Bile salts have been shown to be obligatory for cholesterol absorption, since when bile was prevented from entering the intestinal tract of rats, no cholesterol could be recovered from their lymph although palmitic acid could be absorbed at a reduced rate.[16]

The absorption of other sterols and their effect on cholesterol absorption is a complex and interesting subject. Although Schoenheimer[17] originally reported that plant sterols are not absorbed, evidence has recently accumulated indicating that this is not strictly true. Thus Gould[18] has shown that limited absorption of β-sitosterol can occur amounting to about 10 per cent of cholesterol absorption in rats and human beings. Other investigators have also shown that sterols other than cholesterol are absorbed to a greater or lesser degree.[19–23] It is generally agreed, however, that the saturated sterols and plant sterols are not nearly so well absorbed as is cholesterol. Moreover, inclusion of these sterols in the diet with cholesterol appears to result in a decreased absorption of the latter.[21,24] However, several difficulties are immediately apparent in attempts to control cholesterol absorption and serum cholesterol levels in human patients by this means. First, when substantial amounts of these sterols are fed, they are absorbed to some extent and contribute to serum sterol and to production of atheroma.[19,22] Second, very large amounts of these sterols must be included in the diet before a noticeable effect on absorption is seen. Finally, it has not been proven that regulation of dietary cholesterol or cholesterol absorption has any effect on serum cholesterol in man.

The mechanism of cholesterol absorption has not been thoroughly elucidated. Most of the absorbed cholesterol appears in the lymph, about 70 per cent in the esterified form.[25] Although the poorly absorbed sterols are also not esterified in the intestinal cells,[23] it does not appear that esterification is obligatory in sterol absorption, since even when cholesterol absorption is partially blocked, the proportion esterified remains the same.[26]

The absorption of hydrocarbons such as mineral oil (only if highly emulsified) and the carotenes has been studied to some extent and has again been found to depend on simultaneous absorption of fat, the presence of bile and other factors important in the absorption of fat and cholesterol. However, a complete discussion of this subject is beyond the scope of this review.

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Remarks on the Specificity of Action of Pancreatic Lipase

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I WOULD like to say just a few words about some experiments we made during the past few years in the field of the digestion of lipids. In 1954 when we started this series of experiments,[1] we could show that pancreatic juice, obtained from permanent fistulas in the rat, acting *in vitro* on various natural triglyceride substrates, various seed oils and some animal fats, removed the most saturated fatty acids first. The iodine value of the liberated fatty acids in our experiments was always below the iodine value of the fatty acids of the substrates and increased very regularly during the course of the hydrolysis.

As it had not yet been demonstrated that pancreatic lipase manifested a steric specificity, we thought that the pancreatic lipase might have an affinity for the most saturated fatty acids. Very shortly, the interesting experiments of Borgstrom,[2] Mattson and Beck[3] and Desnuelle and Savary[4] demonstrated that lipase removes first the fatty acids located in the α-α' position (carbons 1 and 3 of the molecule of the glycerol).

We reported for the first time at the meeting in Ghent in 1955,[5] that it was possible to explain the removal of the most saturated fatty acids if, in natural fats and oils, the fatty acids in positions 1 and 3 were generally more saturated than the fatty acids in position 2.

In the meantime Mattson showed that pancreatic extracts acting on lard as glyceride substrate removed the most unsaturated fatty acids first. We recently repeated these experiments with pancreatic juice and lard and found results in good agreements with the results of Mattson. However, we must say that we tried a very large number of other natural fats and oils and that the lard was the only exception to the general rule. We do not know at the moment what explanation can be given for these observations.

We would like to add that we are very impressed by recent experiments of Mattson[6] with synthetic substrates of known structure, and by the difference he observed when pancreatin acts on natural lard or lard after the fatty acids have been rearranged in the triglyceride molecule by hydrolysis and random reesterification. These last experiments, particularly, like those of Savary and co-workers[7] seem very conclusive and speak against the conception of a "chain specificity" of the lipase.

However, it must be recognized that until recently we did not know very much about the structure of the natural triglycerides and we cannot discard completely the hypothesis that the lipase manifests, in addition to its steric specificity, a special affinity for certain kinds of triglyceride substrates present in natural fats and oils.

On the other hand, it is very interesting to note that the pancreatic cholesterol esterase esterified preferentially the most unsaturated fatty acids as we have seen,[8] in good agreement with the experiments of Swell and co-workers.[9]

It may be that in the intestinal tract a certain interrelation exists in the action of the enzymes of the pancreatic juice for esterification and hydrolysis which permit an adequate repartition of the fatty acids between the different lipid constituents. This hypothesis is well supported by results obtained on the composition of the lipid present in the intestinal lumen[10] and in the lymph.[11]

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The Transport of Fatty Acids Across the Cell Wall

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INTENSIVE research efforts of the past few years have led to some understanding of the transport of lipids in the blood and lymph. Their transport across cell walls, an equally complex and interesting subject, has, however, remained completely obscure. Although a complete study of this problem necessarily involves several different lipids in a number of different lipoprotein combinations, the recent information that the most probable form in which fatty acids reach the cell is as the albumin complex prompted us to study this simple lipoprotein first.

For this study we used the Ehrlich mouse ascites tumor because of the ease of making *in vitro* preparations of these cells which could be used in metabolic studies with some confidence that essentially normal reactions were being carried out. Later studies could be performed with other cells such as those from liver or adipose tissue. Experiments were carried out in the conventional Warburg apparatus with washed cells. Substrates used were carboxy-labeled palmitic acid in buffer or as its albumin complex and biosynthetic albumin from ascitic fluid of mice fed phenylalanine-3-C^{14}.

When palmitic acid alone was added to the incubated cells, it was metabolized, since $C^{14}O_2$ appeared in the center wells. However, this oxidation was somewhat irregular and incomplete, ceasing when about 30 per cent of the palmitic acid had been oxidized. After separation of the cells, the supernatant solution retained about 9 per cent of the total C^{14} activity.

When palmitic acid was added as its albumin complex, oxidation, as attested by the appearance of $C^{14}O_2$ in the center well, was regular, and continued for at least 90 minutes. In this case, an average of about 29 per cent of the counts remained in the supernatant solution. When the two substrates were oxidized by the same cells, the differences were even more striking. When the complex containing radioactive albumin was used, no $C^{14}O_2$ appeared in the center well and all the activity remained in the supernatant solution.

From these data, it appears probable that the function of albumin in the fatty acid-albumin complex is one of solubilization and transport to the cell wall and not of aiding in the passage through the wall.

To investigate this aspect further, palmitate or its albumin complex was incubated with normal and KCN-inhibited cells. In the presence of KCN, no oxidation of palmitate occurred, but the proportion of activity in the cell wall was greater than for the normal cells. Evidently active cell metabolism is not necessary for adsorption of fatty acid onto the cell wall.

From the results of these experiments a picture of absorption of fatty acids by cells is becoming clearer. The albumin complex aids in solubilizing and dispersing the fatty acid and in presenting it to the cell wall. At this point, a distribution between the lipid-containing cell surface and the albumin takes place. The fatty acid adsorbed onto the cell wall is then activated and drawn within the cell to be oxidized. The transport of the fatty acid within the cell is still unknown but could possibly be investigated by the same methods.

The transport of other lipids into or out of cells has received some preliminary attention.

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Shapiro, Chowers and Rose[1] have carried out studies in which fat-depleted adipose tissue (from fasted rats) was incubated with various types of lipids, the changes in concentration of which were followed. It was found that fatty acids, simple esters and glycerides were readily taken up by the tissue whereas lecithin and cholesterol esters were not. Stearic acid-1-C^{14} was also absorbed and oxidized by the tissue. James, Lovelock, and Webb,[2] studying the synthesis of fatty acids by red cells, found that fatty acids synthesized *in vitro* are not released into the medium unless serum α-lipoproteins are present in it. This appears to be a distribution in the reverse direction to that of the fatty acid-albumin complex.

From these very preliminary studies, it is evident that this complex and obscure subject is of great importance to the metabolism of lipids by the cell. Many further experiments will be necessary before a clear understanding of these phenomena will be obtained.

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Changes in Tissue Lipids and Tissue Histology Resulting from Essential Fatty Acid Deficiency in Rats

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PROBABLY one of the best ways to study the role of a particular substance in a metabolic process is to induce the deficiency condition in animals. Much of the information concerning the function of essential fatty acids in nutrition and in the regulation of cholesterol metabolism has been derived using this procedure.

Contribution #450 from the Harry J. Deuel, Jr. Laboratories, Dept. of Biochemistry and Nutrition, University of Southern California, School of Medicine, Los Angeles.

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Essential fatty acid deficiency can be induced in rats by feeding a diet containing no added fat, which is essentially a high carbohydrate diet. Animals placed on this diet at weaning become deficient in essential fatty acids in about 12 weeks. The presence of certain completely saturated fats in the diet, e.g., hydrogenated coconut oil, hastens the appearance of some of the essential fatty acid deficiency symptoms.[1]

Essential fatty acid-deficient animals de-

Fig. 1. Section of epididymis from control animals. Note the sperm collected in the lumina of the ducts.

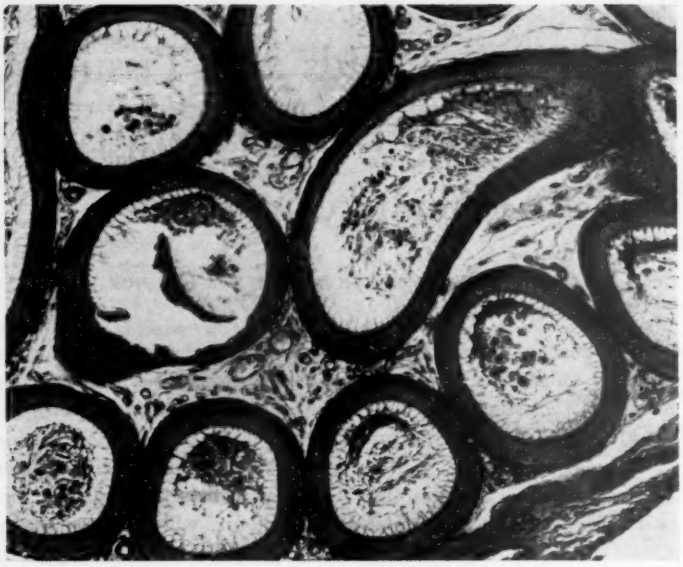

Fig. 2. Section of epididymis from essential fatty acid-deficient rat. Note the absence of sperms and the presence of cellular debris in the lumina of the ducts.

velop a syndrome characterized by scaliness of the paws and tail,[2] capillary fragility,[3] increased skin permeability,[4] alopecia, ocular lesions, and priapism. Growth is poor,[5] and there is interference with reproductive ability in both male and female rats.

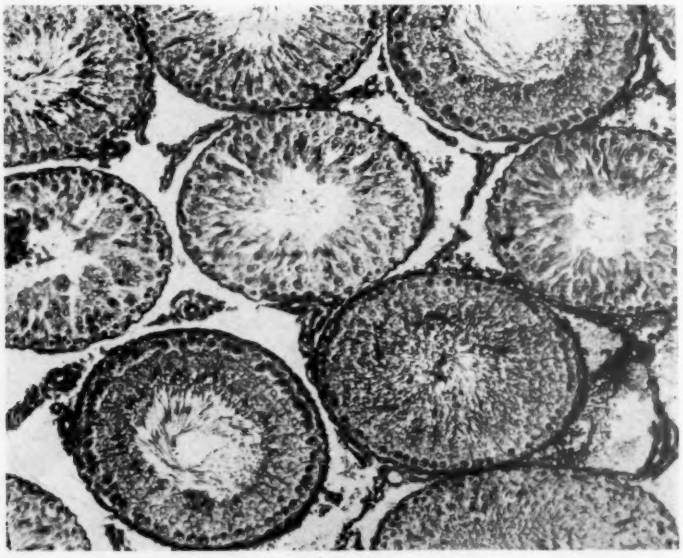

Fig. 3. Section of testes from control animal. Note that the tubules contain active maturing spermatogonial cells undergoing spermatogenesis with sperms in the lumina.

GONADAL TISSUE

The loss of fertility in male rats on fat-free diets is easily explained by a histologic examination of gonadal tissue of the male animals. This was originally observed by Evans *et al.*[6,7] and has been confirmed by observations made recently in our laboratories. In the essential fatty acid-deficient animal there is impairment of spermatogenic development. In the epididymis of normal animals, the lumina of the ducts are filled with mature sperm (Fig. 1), but there is an almost complete loss of sperm in the lumina of the ducts of the deficient animals (Fig. 2). In the testes themselves a depletion of essential fatty acid produces a degeneration of the tubules and a loss of maturation of the primary spermatogonial cells as compared with the control (Fig. 3 and 4). The addition of small amounts of essential fatty acid to the diet of male rats so affected can cure, as well as prevent, this condition.

The loss of reproductive ability in the male rats is reflected in the histologic sections of the pituitary gland. Figure 5 is a section of the anterior pituitary of the control animals stained for gonadotrophs. The function of gonadotrophs is to secrete follicular stimulating hormones for the stimulation of testicular development. In the animal on a fat-free diet, where there is testicular degeneration, the testes are unable to produce sperm, thus stimulating an increase in gonadotrophs in the pituitary gland, yielding the picture seen in Figure 6.

In the female rat (Table I), it can be seen that although essential fatty acids are not required for conception, they are required for the continuance of the development of the fetuses, and for parturition.[8]

BONE

The poor growth of rats fed a fat-deficient diet is reflected not only by the absence of adipose tissue but also in histologic changes in the bone. The cessation of growth and the subsequent plateau in weight of these animals is caused by a reduction in the number of proliferating cells. As an example, in the

TABLE I

The Effect of Different Levels of Linoleate on Pregnancy, Survival, and Growth of Young from Females on a Fat-Free Diet

Category	11	12	13	14	15	16	17
Linoleate fed daily (mg)	0	2.5	5.0	10	20	40	80
Females bred	16	15	16	16	16	16	16
Litters cast, per cent	87	93	87	100	100	94	100
Litter (at birth)							
Total number of rats	69	93	96	111	120	108	115
Average number per litter	4.9	6.6	6.8	6.9	7.5	7.2	7.2
Litter (at 3 days)							
Total number of rats	0	0	0	14	68	79	99
Total litters represented	—	—	—	3	11	15	14
Average weight per rat (g)	—	—	—	5.0	4.9	5.6	6.5
Litter (at 21 days)							
Total number of rats	0	0	0	—	44	58	77
Total litters represented	—	—	—	—	8	10	13
Average weight per rat (g)	—	—	—	—	22.9	25.7	29.2
Mortality (0–3 days)							
Total	69	93	96	97	52	29	16
Per cent	100	100	100	87	43	27	14
Mortality (3–21 days)*							
Total	—	—	—	13	21	11	12
Per cent	—	—	—	100	32	16	13

* Litters were cut to seven at three days.

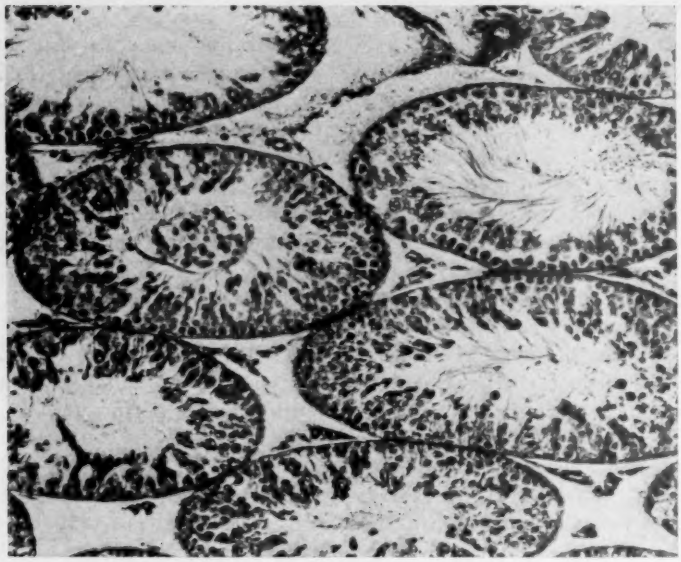

Fig. 4. Section of testes from an essential fatty acid-deficient animal. The tubules are undergoing degeneration characterized by loss of primary and secondary spermatocytes, sloughing off from tubular wall, cellular debris in lumina and absence of sperm in lumina.

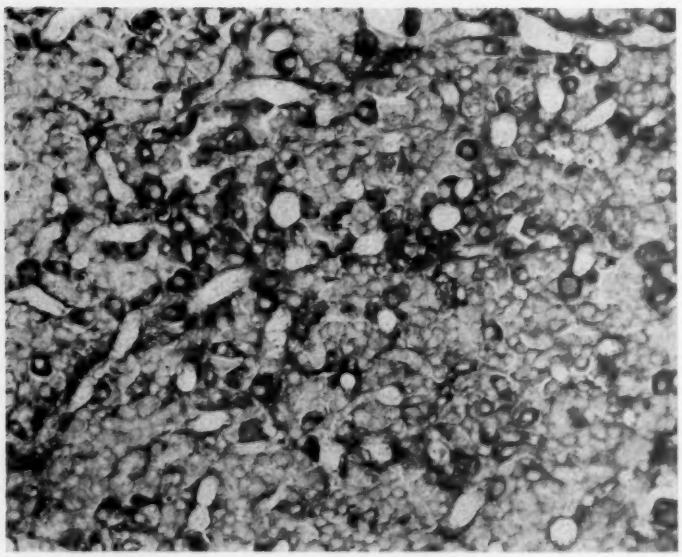

Fig. 5. Section of anterior pituitary stained by periodic acid-Schiff method for the demonstration of basophilic gonadotrophs (control animal).

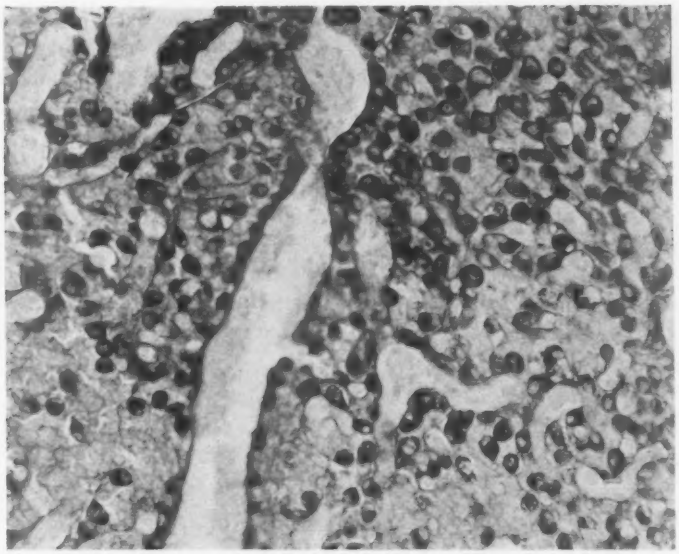

Fig. 6. Section of anterior pituitary stained by periodic acid-Schiff method for the demonstration of basophilic gonadotrophs (essential fatty acid-deficient animal). Note an increase in number of gonadotrophs as compared with control.

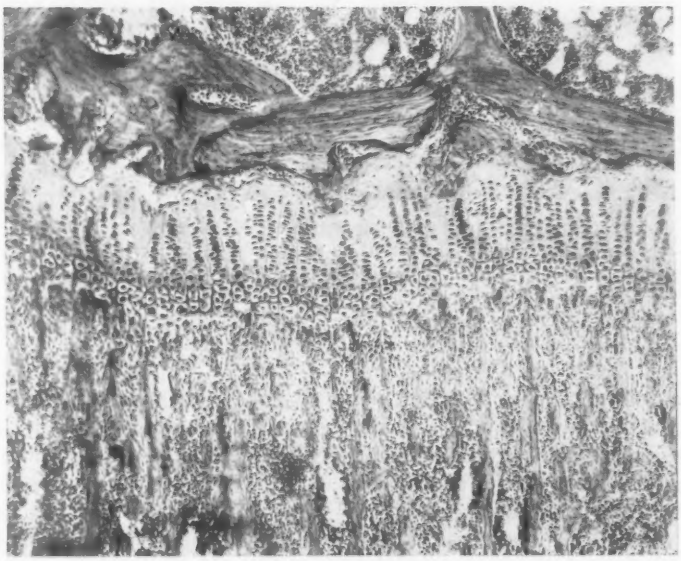

Fig. 7. Section of tibia, control animal. Note the four zones of the cartilage, especially the proliferating region which is characterized by the parallel columns of cells. The trabeculae of the diaphysis are long and slender.

proximal head of the tibia, normally there is a wide section composed of cartilage cells in which cell division occurs (Fig. 7). This cell division is responsible for the growth of the bone. In the fat-deficient animal, at the diaphyseal border there is a thin layer of bone sealing off the epiphyseal plate; and in the diaphysis there is a loss of bone cells, and a replacement of myeloid elements by adipose tissue (Fig. 8).

Degenerative changes resulting from essential fatty acid deficiency are also apparent in histologic sections of the alveolar bone. This bone in the control animal consists of a dense alveolar bone proper and an inner thick trabeculated spongiosa (Fig. 9). In the animal on the fat-free diet, there is an extensive osteoporosis with an increase of fatty tissue in the marrow space (Fig. 10).

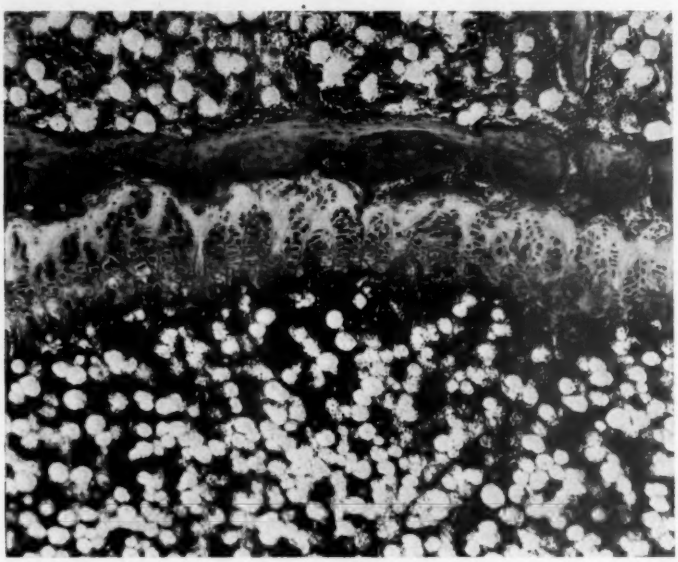

Fig. 8. Section of tibia, essential fatty acid-deficient animal. Note the decrease in the number of cells in all regions, especially the proliferating region, with a relative increase in the amount of intercellular matrix. There is a loss of trabeculation projecting into the diaphysis and sealing off by a thin layer of bone, preventing further growth. In addition, there is a replacement of myeloid elements by adipose tissue.

LIVER

In the response of rats to essential fatty acid deficiency a sex difference is apparent. Males are much more susceptible to the ill effects resulting from essential fatty acid deficiency than are female rats, indicating perhaps a difference in requirement for these acids. A further result of essential fatty acid, deficiency in the male rat, which has not been observed in the female animals, is the increased concentration of cholesterol in the liver and adrenal gland and a reduced cholesterol content in plasma (Table II).[9] The increased cholesterol concentration in the liver is accounted for as an increase in esterified cholesterol. The type of cholesterol esters deposited in the liver in essential fatty acid-deficient animals is different from those which occur normally. Whereas in the normal liver, 23 per cent of the fatty acids in combination with cholesterol are polyenoic, in the deficient animal, cholesterol is combined with more saturated fatty acids (Table III).[10]

The liver of essential fatty acid-deficient animals is milky in color and fragile in texture. Examination of histologic sections of liver

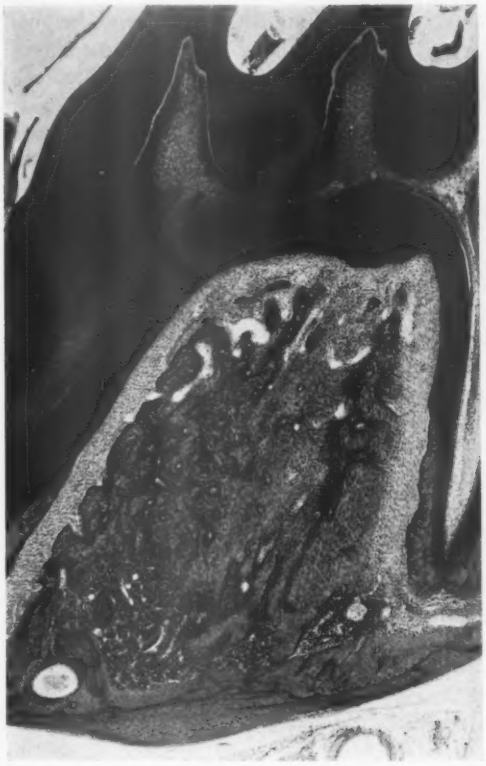

Fig. 9. Section of alveolar bone from control animal. Note dense bone in the spongiosa.

Fig. 10. Section of alveolar bone from essential fatty acid-deficient animal. Note osteoporosis of spongiosa.

TABLE II

The Effect of a Diet Deficient in Fat on Cholesterol Levels in Various Organs of the Rat

Diet	Number of rats	*mg* Cholesterol/*g*		*mg* Cholesterol of 100 *ml* Plasma
		Liver	Adrenal	
12.5% fat	10	2.04	35.4	65.6
	9	2.08	35.3	63.2
Fat-free (vitamin-test casein)	8	3.15	48.9	38.4
	7	4.06	50.4	50.4
Fat-free (commercial casein)	10	4.72	49.3	41.1
	9	4.24	46.2	44.9

TABLE III

The Effect of Diet on the Fatty Acid Composition of Cholesterol Esters in Rat Liver

Fatty acids	Diet		
	15% Cottonseed oil	Fat-free	Fat-free + linoleate
Saturated	17	27	19
Monoethenoid	60	73	58
Diethenoid	18	—	18
Polyethenoid	5	—	5
Iodine value	104	67	100

tissue substantiated chemical analyses and gross observations. In the control animals, the liver lobules appear well organized, and there is no evidence of fatty infiltration. There is a normal granular appearance of the cytoplasm (Fig. 11). On the other hand, liver sections from animals fed fat-free diets in general, show various degrees of fat infiltration as compared with sections obtained from animals fed the control diet (Fig. 12). This fat infiltration is irregularly distributed, with a

Fig. 11. Section of liver from control animal stained with Sudan black for the demonstration of fat. Note the apparent absence of heavy fat deposits.

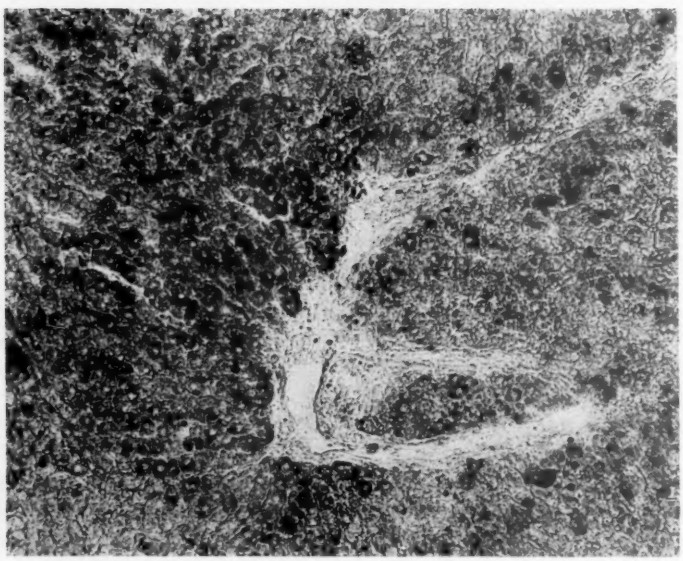

Fig. 12. Section of liver from essential fatty acid-deficient animal. Note the heavy deposits of fat droplets in the liver cells radiating from the periphery towards the center of the lobule.

high concentration of fat-droplets in liver cells located at the periphery adjacent to the portal area and a progressively lesser involvement toward the center of the lobule. Fat vacuoles of varying size are visible in the cytoplasm of the hepatic cells.

Since the accumulation of fat and cholesterol in the liver might have been due to excessive synthesis of these substances, studies on cholesterol biosynthesis from labeled acetate were undertaken using liver slices. Rather than increased synthesis of cholesterol, there is a marked decrease in incorporation of acetate C^{14} into cholesterol in the fat-deficient animal (Table IV).[11] In the animals fed the fat-free diet supplemented with linoleate, synthesis of cholesterol is essentially normal. It is therefore theorized that the accumulation of cholesterol in the liver is due to an impairment in the systems responsible for cholesterol ester mobilization and transport.

Sections of liver were also stained for the demonstration of glycogen. In the sections from animals on the control diet, a heavy deposit of glycogen is apparent throughout the liver cells (Fig. 13). In the livers of animals on the fat-free diet, there is very little demonstrable glycogen—a few glycogen granules appear in the cytoplasm near the central veins (Fig. 14).

TABLE IV

Cholesterol Concentration and Incorporation of Acetate-1-C^{14} into Cholesterol in the Liver of Rats on Various Diets

	Diet	Weeks on diet	Total cholesterol *mg/g*	% total counts incorporated	Counts/min/g tissue
I	15% Cottonseed oil	1	2.39	4.01	7204 ± 892
		4	2.47	4.40	7946 ± 286
		16	2.51	4.14	7482 ± 200
II	Fat-free	1	3.70	0.33	587 ± 73
		4	3.74	0.78	1408 ± 106
		16	4.39	0.51	914 ± 42
III	Fat-free + 100 mg EFA/rat/day	1	2.79	3.45	6212 ± 136
		4	2.81	3.78	6791 ± 214
		16	2.69	4.17	7502 ± 307

EFA = essential fatty acid.

In the liver of animals on the fat-free diet there is also an increased deposition of iron. This may be the result of iron being deposited at a rate faster than the appropriate amount

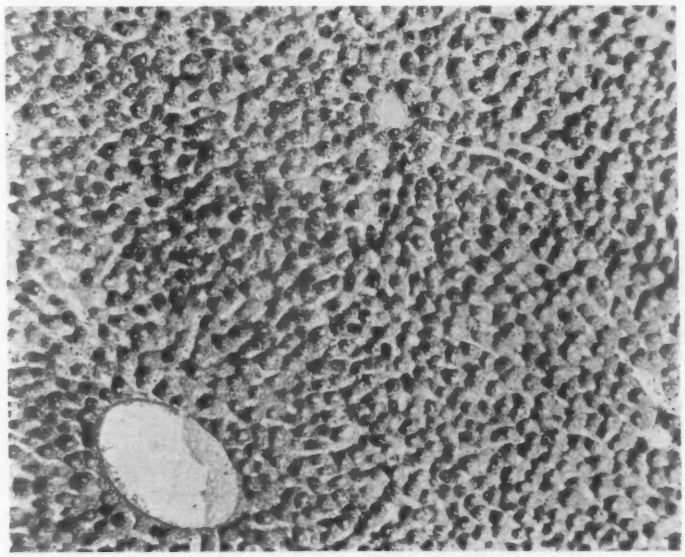

Fig. 13. Section of liver from control animal stained by periodic acid-Schiff method for demonstration of glycogen. Note the heavy deposits.

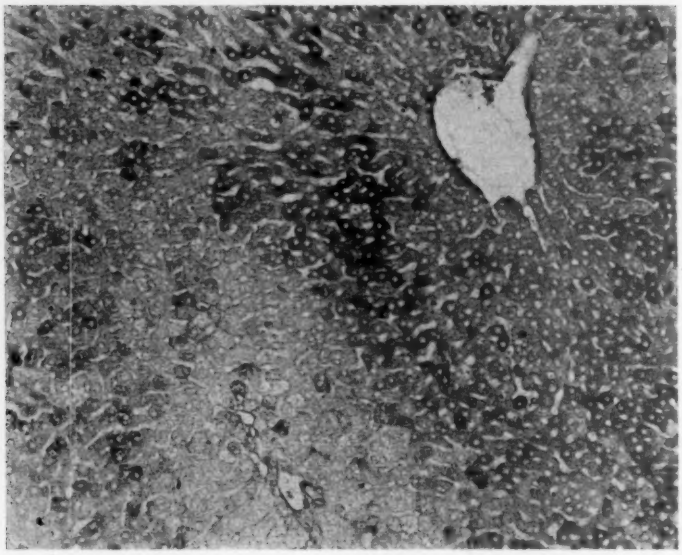

Fig. 14. Section of liver from essential fatty acid-deficient rat stained by periodic acid-Schiff method. Note the marked loss of demonstrable glycogen in the liver.

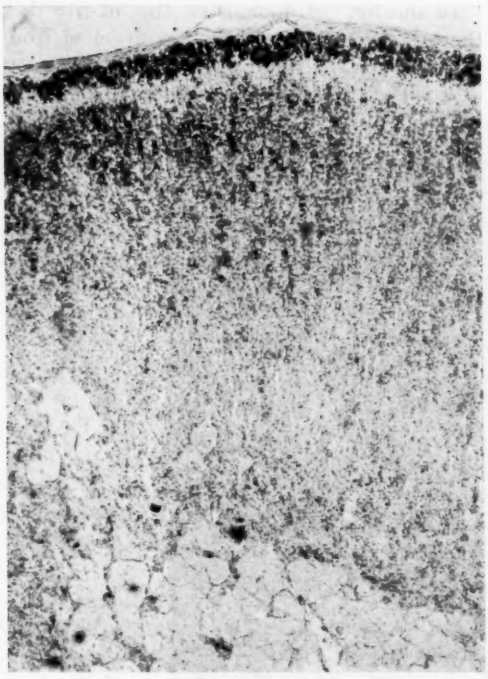

Fig. 15. Section of adrenal gland of control animal stained with Sudan black. Note the distribution of fat in all cortical layers, and the wide cortex.

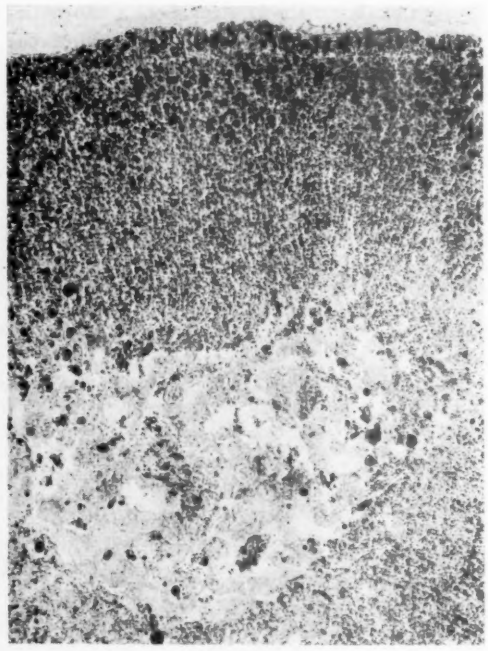

Fig. 16. Section of adrenal gland of essential fatty acid-deficient animal stained with Sudan black. There is a decrease in the width of both fasciculata and reticularis, but an increase in the amount of the fat droplets.

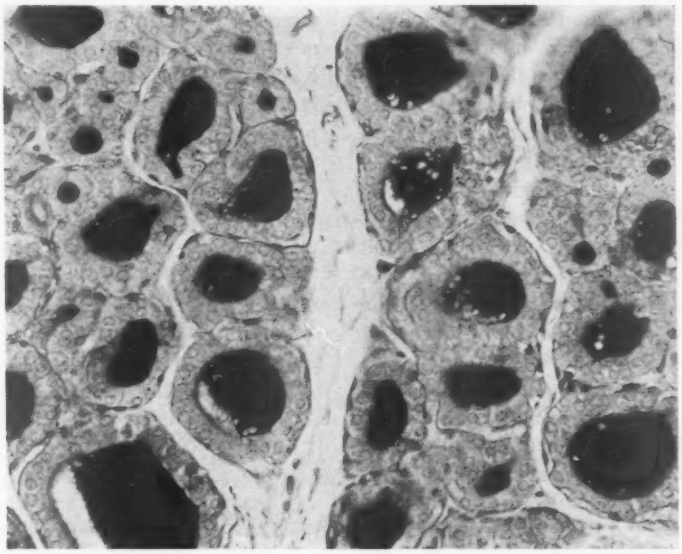

Fig. 17. Section of thyroid of control animal stained by periodic acid-Schiff method. Note the well formed follicles with colloid in their lumina. Thyroglobulin droplets are evident in the cytoplasm of the follicular cells.

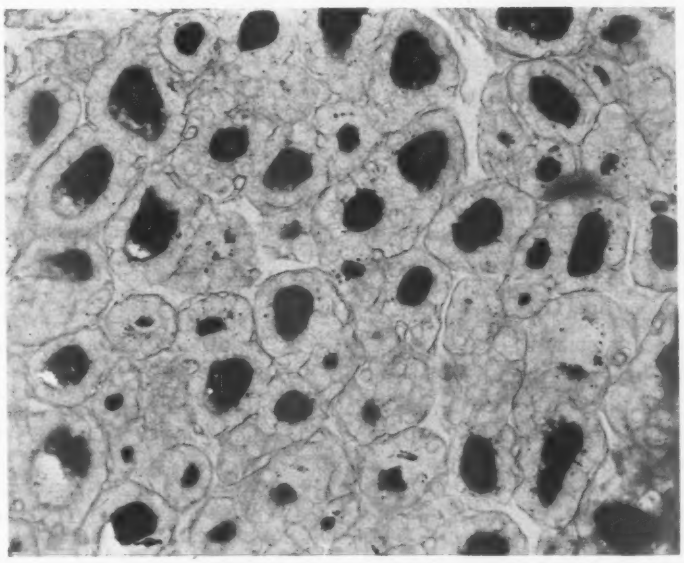

Fig. 18. Section of thyroid from an essential fatty acid-deficient animal stained by periodic acid-Schiff method. Note decrease in amount of stored colloid. Also observe the increase in thyroglobulin droplets in cytoplasm of follicular cells.

of apoferritin can be synthesized, as a consequence of which the ferric hydroxide phosphate units polymerize into large stainable granules. Hemochromatosis has also been reported in various pathologic and dietary conditions, e.g., anemia and diets low in protein.

ADRENAL

This decrease in glycogen in the liver could be a reflection of decreased activity of the adrenal gland. In the adrenal gland of the animal on the fat-free diet, there is evidence of atrophy with a decrease in width of the cortical layers, fasciculata and reticularis, as compared with the wide cortex in the control section (Fig. 15 and 16). A decrease in cortical activity causes a decrease in gluconeogenesis. The increase in the amount and size of the fat droplets in the adrenal gland of the essential fatty acid-deficient rat substantiates the chemical analyses previously noted (Table II).

THYROID

Sections of the thyroid gland show the appearance of a hyperactive gland in essential fatty acid-deficiency. In the normal gland, there is a heavy deposit of colloid containing iodine, and well shaped spherical follicles (Fig. 17). In the fat-deficient animal, follicles are smaller in size (Fig. 18). There is less stored colloid; many of the follicles are collapsed and contain colloid droplets in the cytoplasm.

Both the chemical and histologic abnormalities observed here are not evident when rats are fed a fat-free diet supplemented with linoleate esters. However, although the essentiality of essential fatty acids has been proven for the rat and several other species of animals, the absolute necessity for essential fatty acids has not been proven in man.* It is known that certain human skin diseases are helped by a diet supplemented with essential fatty acids. Diets containing vegetable oils rich in essential fatty acids are now being advised by many investigators for the reduction of elevated serum cholesterol levels. Although it is improbable that a human dietary regimen is entirely deficient in essential fatty acids, it is possible that the requirements for these fatty acids are elevated in certain disease conditions.

* *Editor's Note:* On the basis of the data presented by Dr. Hansen, perhaps one can say that "the absolute necessity for essential fatty acids *has* been proven in man."

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The Problem of the Essential Fatty Acids in Relation to Human Nutrition

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IN CONTRADISTINCTION to most clinicians who presently are concerned with the problem of the dietary significance of essential fatty acids our interest has been of long duration. As pediatricians, quite naturally, our chief objective has been to attempt to define the role of linoleic acid in infant feeding and it may be said that our approach has been conservative. To pursue our endeavors it has been necessary to carry on considerable work with chemical methods as well as with experimental animals, which studies have been carried on in collaboration with Hilda F. Wiese. A number of persons have assisted in these investigations (acknowledgments of which may be found in *Pediatrics* 21: 494, 1958).

Shortly after the turn of the century it was the accepted clinical practice to remove fat from the diet of the child with eczema. Seldom, if ever, in the author's experience was this procedure of benefit to the infant. Because of this apparent therapeutic failure, the idea was entertained that dietary fat actually may be advantageous to the child. After the observations of Burr and Burr on the essentiality of certain fatty acids, there was distinct support of this idea. It was observed that a number of infants with intractable eczema responded favorably when fat rich in the unsaturated fatty acids was added to their diet; hence, it seemed logical to pursue studies in the field of lipid metabolism. A number of years were spent in developing chemical methods which would allow one to measure specific unsaturated fatty acids in the blood serum of small infants. In working with William R. Wilson at Yale it was our good fortune to develop a semimicrogravimetric technic wherein it was possible to separate the saponifiable from the nonsaponifiable lipid fractions. For example, in so doing, it was no longer necessary to make assumptions regarding the composition of the material which was separated with cholesterol in the unsaponifiable portions of the blood serum. Extension of the basic principles of this procedure to the study of the phospholipid fraction revealed that the average molecular weight of the phospholipid fatty acids was higher than that which had been assumed. It was also demonstrated that the average iodine number of the cholesterol ester fatty acids was higher than the fatty acids present as glycerides and phospholipids.

It was our good fortune to find that young dogs were especially suitable animals for study of the dietary significance of the essential fatty acids. For example, when young puppies were placed on a diet very low in fat it was observed that within a few weeks there developed a thickening of the skin, desquamation, later loss of hair, with oozing of the feet and running of the ears. Even after the animals had been in despicably bad shape for several years, it was possible to restore the animals to complete normalcy when the diet contained linoleic acid in adequate amounts. Spectacular changes also were demonstrated in the histologic structure of the skin. Both the gross and the microscopic findings could be correlated with the level of linoleic acid in the diet. More significant, however, was that the above features could be correlated with the level of the 2-, 3-, and 4-double bond fatty acids of the blood

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serum. With the development of the alkaline isomerization method of chemical analysis, the results of which correlated so well with the gross and microscopic appearance of the skin, it was felt that one was in the position of attempting to learn something definitive about the role of linoleic acid in the diet of infants.

The use of low-fat diets in the feeding of infants seemed fully justified, first, because a number of pediatricians considered this to be the method of choice for feeding premature and small infants and second, the experience of some observers indicated satisfactory progress with this type of dietary regimen. Thanks to grants from the United States Department of Agriculture, Institute of Home Economics, Human Nutrition Research Division, Washington, D. C.; Baker Laboratories, Inc., Cleveland, Ohio; and Gerber Products Company, Fremont, Michigan, it has been possible to carry on rather extensive clinical studies in infant feeding wherein the only variable has been the substitution of carbohydrate for fat. Only preliminary reports have been made inasmuch as the studies are still under way; however, we may mention a few features which have been demonstrated.

Serum Lipid Findings: Good correlations have been found between the linoleic acid in the diet of infants and the blood serum levels of the di-, tri-, and tetraenoic acids. Because of the variations which occur in the level of the total fat in the blood from individual to individual as well as in the same individual from time to time it has been found more meaningful to express the values for the di-, tri-, and tetraenoic acid in terms of "per cent of the total fatty acids" rather than in milligrams per cent. This also holds for the fatty acids present as phospholipids, cholesterol esters, as well as glycerides. With a low fat diet the most marked changes are decreases in both the di- and tetraenoic acid levels of the blood serum. On the other hand, the trienoic acid level increases. When the diet contains liberal quantities of linoleic acid there is a distinctly higher level of the dienoic acid in the blood serum and to a lesser extent also of the tetraenoic acid, whereas the trienoic acid values drop. Dienoic acid seems to be the chief fatty acid present as cholesterol ester. The greatest change in the trienoic acid value occurs in the phospholipid fraction although the levels of di- and tetraenoic acids in this acetone-insoluble fraction also vary directly with the linoleic acid in the diet.

Clinical Evidence of Fat Deficiency in Infants: Rather remarkable has been the observation that young infants fed on diets low in fat frequently develop definite changes in the skin consisting of dryness and desquamation, denudation, and exuding in the body folds. It has been demonstrated that this skin abnormality disappears completely within a week to ten days after adding 2 per cent of the calories as linoleic acid to the diet or changing to a milk mixture containing liberal amounts of linoleic acid. It may be conjectured that this observation is of some clinical significance because some 20 years ago in a rather comprehensive clinical study it was observed that eczematous skin eruptions occurred seven times more frequently in artificially fed infants than in infants who were entirely breast fed. The speculation has been made that contributing to this phenomenon is the fact that mother's milk contains 4 to 5 per cent of the calories as linoleic acid compared to about 1 per cent of the calories in the usual cow's milk formulas used in the artificial feeding of infants. In other words, in the consideration of factors which are concerned with maintenance of the integrity of the skin, dietary linoleic acid is of distinct significance.

Caloric Consumption in Relation to Linoleic Acid in the Diet: In view of the many studies which have been carried out to quantitate the caloric requirements of infants it seems rather remarkable that observations have been made which indicate that infants given diets extremely low in fat tend to consume a greater number of calories than when their diet contains fat. It has been found that in babies given diets extremely low in fat the addition of 1 per cent of the calories as linoleic acid decreases caloric consumption. Perhaps it should not be so surprising that dietary linoleic acid affects caloric consumption when one realizes that one of the earliest observations made on rats maintained on fat deficient diets was growth failure. Also, it has been found that young puppies de-

velop severe inanition when maintained on fat-deficient diets in spite of iso-caloric consumption of food compared with healthy control animals. The late Dr. William R. Brown maintained himself on a diet extremely low in fat for a period of six months. Metabolic studies indicated that his responses were the same as those found in fat deficient rats. Unfortunately, the enzymatic mechanisms concerned with caloric efficiency, as yet, are not known.

Finally, one may say that a feature which is rather disconcerting to the speaker is the likelihood of continued confusion in this field by failure to name specific fatty acids when speaking of dietary "essential fatty acids." Too often a rather loose term is used to connote more than one unsaturated linkage in the fatty acid chain. It is believed that the development of simpler methods of determination will lead to a better understanding of the exact role played by linoleic acid in infant nutrition. However, alkaline conjugation methods with spectrographic readings for specific fatty acids have been found to give satisfactory results in relation to gross and histologic features which are indicative of the dietary need for the essential fatty acids. It is further conjectured that when the dietary as well as metabolic significance of linoleic acid is better understood in infant feeding the groundwork will be laid for a real understanding of the role played by specific fatty acids in the nutrition of adult human subjects.

Essential Fatty Acids and the Problem of Atherosclerosis

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IN 1950 we had occasion to carry out studies designed to evaluate the effects of certain hormonal entities upon fatty acid oxidation. As part of this study we placed patients upon diets containing very large amounts of fat. Since vegetable fat is liquid at room temperature, and emulsions made from liquid fat are easier to work with, vegetable fat was selected for this study. In the course of this work we noted a very profound decrease in the level of plasma cholesterol and phospholipids. Initially, it was felt that the decrease was the result of hormonal agents which were co-administered, but subsequent studies in which no hormones were used indicated that the effect could be produced essentially at will by inclusion of adequate amounts of a variety of vegetable fats.[1–4]

These observations, which were first confirmed by Ahrens,[5] and since by many other workers, raised the question as to whether this effect of vegetable fat was referable to the *presence* of something or the *absence* of something. When equal amounts of animal fat were substituted for the vegetable fat, a prompt rise in the cholesterol and other lipids occurred. However, when very large amounts of cholesterol were added to the vegetable fat diet, little or no increase in the level of the plasma lipids was noted. It therefore seemed proper to say that this effect was referable not merely to the absence of cholesterol.

Vegetable fats contain sterols other than cholesterol as well as a variety of phospholipids. Accordingly, for several years in the period after 1950, studies were undertaken in which vegetable sterols and related substances were administered under controlled conditions. In a few instances some decrease in the level of plasma lipids was noted. However, these changes were inconstant and usually of relatively slight degree. Much the same statement applies to the administration of phospholipids of vegetable origin, although in a few instances the administration of vegetable phosphatides did produce a very significant decrease in the level of plasma lipids. Subsequent studies have shown that those phospholipid preparations which produced such an effect were compounds containing relatively large amounts of polyunsaturated fatty acids.

This then left us with essentially one probable explanation—that specific fatty acids present in vegetable fats were responsible for the decrease in plasma lipids.[6]

During the past one and one-half years, studies have been carried out using purified preparations of ethyl oleate, ethyl linoleate, tri-olein, and tri-linolein. These studies have demonstrated beyond any reasonable doubt that the administration of diets con-

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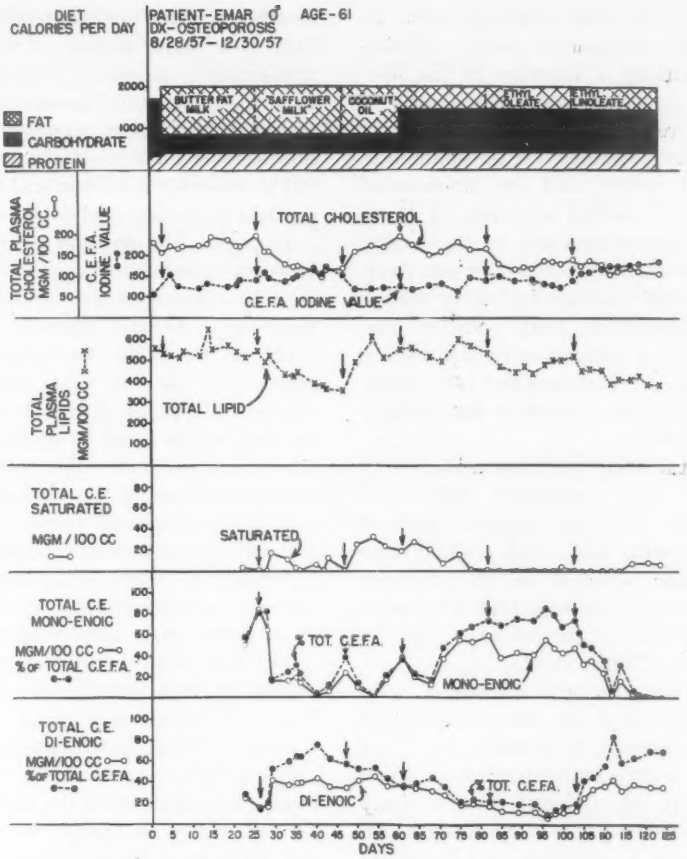

Fig. 1. Effects of different fats upon plasma lipids. The monoenoic and dienoic acid content of the cholesterol esters mirrors the type of fat fed,* particularly in the case of ethyl oleate and ethyl linoleate.

taining purified linoleic acid preparations as compared to diets containing saturated fats, purified oleate or fat-free diets will produce a decrease in plasma lipids of at least as great a magnitude as will vegetable fats containing equal or greater amounts of linoleic acid.[7]

In Figure 1 is shown a portion of a study in patient EMAR in which oleate is compared with linoleate. The plasma lipid levels during linoleate administration are significantly lower than those observed during oleate ingestion. Also, the mono- and dienoic acid content of the plasma cholesterol esters mirrors the type of fatty acid which is being administered.

These observations raise two major questions: First, what is the mechanism of this effect? And second, is the lowering of plasma lipids noted under these conditions associated with a desirable effect on atherogenesis or upon existing atherosclerosis?

The mechanism of the effect is still unclear. As a working hypothesis, we would advance the following proposal: Fatty acid is the prime fuel of the body. It is probable that 70 per cent or possibly more of the total energy expended in a 24 hour period is obtained from the combustion of fatty acids. Consequently,

* Safflower milk—"Saf-Lac" (Carnation Co.).

a large amount of such material must be solubilized and transported daily. Solubilization is essentially a function of the lipoproteins.

Fatty acids are transported in four types of compounds: the glycerides, the phospholipids, the cholesterol esters, and the unesterified fatty acids. The precise sequence of "passage" of fatty acids from one of these compounds to the next is by no means clear. We postulate that common fuel fatty acid is carried in the glycerides; that, in order to be passed on for final utilization in the tissues, perhaps by way of the unesterified fatty acids, the fuel fatty acids are carried by the lecithin-type phospholipids.

Lecithin contains one polyunsaturated fatty acid and one fuel (saturated) acid. The former, together with one molecule each of glycerin, phosphoric acid, and choline, represent the "basic skeleton" of the phosphatide which transports the saturated fatty acid. The saturated acid is turned over rapidly, the "basic skeleton" quite slowly.

Cholesterol esters serve as a donor of polyunsaturated fatty acid for new phospholipid formation. So long as all of these factors are in equilibrium, with particular reference to an adequate supply of polyunsaturated fatty acid, the rate of formation of cholesterol and phospholipid is at a minimum and the blood level is low.

If essential fatty acid is in short supply, there is an increased rate of cholesterol and phospholipid formation, an increased level of both materials in the plasma in an effort to make up in quantity what is lacking in quality, and, consequently, an increased tendency to abnormal deposition of these compounds in tissues such as the intima of arteries. Supply of an adequate amount of linoleic acid reverses this entire trend. We again emphasize: this is a working hypothesis (vintage of 1957–58) which is being subjected to progressive experimental evaluation.

We are aware that some reported data indicate that there is an increased excretion of cholic acid and an increased excretion of sterols in the stool when unsaturated fats are fed.[8] These data are interpreted as indicating increased excretion of cholesterol and its metabolites as a major avenue of unsaturated-fatty acid-induced cholesterol lowering. In our own experience, the sterol excretion is highly inconstant. Increase may occur with fats which do not decrease the level of plasma lipids and may fail to occur when linoleic acid is fed. We have no data regarding cholic acid excretion.

Regarding the question as to the relationship between atherogenesis or the resorption of existing atherosclerosis, on the one hand, and lowering of plasma lipids by essential fatty acids, on the other, two parts of a dilemma exist. The first is that, because of major species differences, no laboratory animal can be used satisfactorily to supply data regarding the human subject. The second part of the dilemma relates to the obvious fact that one cannot examine and re-examine the interior of arteries in the human subject.

During the course of the last three years we have had an opportunity to observe a variety of patients with known atherosclerosis. In individuals with peripheral vascular disease associated with intermittent claudication, we have seen highly significant improvement in a number of such patients during the intake of polyunsaturated fatty acids. Since this group represents a segment of the population that can be objectively evaluated, we feel that if the trend proves to be consistent and predictable, one would have increasing reason to believe that the lowering of plasma lipids induced by linoleic acid is, in fact, associated with favorable effects upon either formation of plaques or resorption of existing plaques. The recent *in vitro* observations of Rutstein seem to be compatible with this concept.[9]

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The Use of Diets Containing Large Amounts of Linoleic Acid

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THE study of certain aspects of lipid metabolism has been the major interest of this Institute for the last eight years. During this time, patients on constant-intake balance studies have been fed various formulae, either through polyethylene tubes, or orally, as described in an earlier paper.[1] Evaluation of the plasma lipid patterns of these patients has led to increasing emphasis on the inclusion of polyunsaturated fats in both formula and mixed diets in ambulatory and hospitalized patients with certain diseases, particularly atherosclerosis and diabetes. In this paper we present the details of the diets used in our studies.

The mixed diets presently in use (Tables I and II) contain, per 2,000 cal, approximately 80 g protein, 170 to 200 g carbohydrate and 100 to 115 g of fat, including approximately 58 g of linoleic acid.

The group I diet contains no animal fat. Protein is supplied by nonfat milk, nuts, cereals and vegetables.

The group II diet differs from group I by including fish, fowl and liver, with a slight reduction in nonfat milk protein.

The group III diet adds to the group II diet small quantities of lean beef, veal, lamb, lean ham, and eggs, with a further reduction of nonfat milk protein.

If the diets are to be followed successfully, the patients must be adequately motivated and properly instructed. The psychologic problems are essentially the same as those encountered in the routine care of diabetics.

In some individuals with advanced atherosclerosis, even the group I diet fails to bring the plasma cholesterol and other lipids to normal levels (below 200 mg per 100 ml). Such patients are given a "safflower-milk" diet (Table III) consisting of an emulsion of 300 g "Saf-Lac"† powder (1,700 cal) with sufficient low carbohydrate vegetables and fresh fruit to bring the intake to 2,000 calories. This diet provides approximately 85 to 90 g. of linoleic acid.

References 2–10 list sources of information regarding fatty acid composition of various foods.

GENERAL INSTRUCTIONS

Fruits and Vegetables

Consider banana, red and white grapes and sweetened canned fruits as 20 per cent fruits, and all other fruits, either fresh or canned without sugar, as 10 per cent fruits. Consider potato, cooked dried beans, corn and cooked rice as 20 per cent vegetables; and beets, carrots, peas, onion and winter squash as 7 per cent vegetables. Other vegetables are unclassified.

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† "Saf-Lac," a modified milk preparation which is free of butter-fat and contains 40 per cent safflower oil. A dried powder, it readily reconstitutes when water is added. Manufactured by the Carnation Co.

TABLE I

2,000 Calorie Diets for Achieving and Maintaining Plasma Cholesterol below 180 mg/100 cc

Food	Group I		Group II		Group III	
	Household measure	g	Household measure	g	Household measure	g
†Nonfat milk solids or liquid skimmed milk	3/4 cup or 1 qt	90	1/2 cup or 2 1/2 cups	60	3 tbsp or 1 cup	25
Low-calorie cottage cheese	2/3 cup	130	1/2 cup	100	1/2 cup*	100
Fish, fowl, liver	None	None	4 oz	120	4 oz or	120 or
Lean beef and veal round, lamb leg, lean ham	None	None	None	None	4 oz	120
†Oil—safflower, corn, cottonseed, soy	1/4 cup or 4 tbsp	60 ml	Same as group I		Same as group I	
Nuts—at least 50% walnuts	2/3 cup	65	Same as group I		Same as group I	
Cooked whole-grain cereal	1/2 cup	100	Same as group I		Same as group I	
Fruits—20% (or) 10%	1 1/2 cups or 3 cups	300 or 600	Same as group I		Same as group I	
Vegetables—20% (or) 7%	1 cup or 3 cups	200 or 600	1/2 cup or 1 1/2 cups	100 or 300	1/2 cup or 1 1/2 cups	100 or 300
Unclassified	As desired cooked, raw		As desired		As desired	
Low-fat bread	1 slice	25	2 slices	50	2 slices	50
Total	Protein	78	Protein	87	Protein	73
	Fat	100	Fat	107	Fat	118
	Carbohydrate	209	Carbohydrate	188	Carbohydrate	170
	Linoleic acid	58 (approx)	Linoleic acid	58 (approx)	Linoleic acid	58 (approx)

* Three times weekly, one egg may be substituted for 1/4 cup of cottage cheese.

† 1 cup (16 tablespoonfuls) of "Saf-Lac" is equivalent to 3/4 cup nonfat milk solids and 3 tablespoonfuls of oil.

Low-Fat Breads

These include sweet or sour French bread, Milwaukee pumpernickel, English muffins, and Ry-Krisp.

Oils

Safflower, corn, cottonseed and soy oils may be used in cooking, as a salad dressing, added to cereals, or emulsified with skimmed milk. *Be sure that all oil is used* and none is left in the bowl or on cooking utensils.

Nuts

Nuts add variety. They may be eaten as such, blended with milk, or added to prepared dishes. At least half the nuts eaten should be walnuts, which are particularly rich in linoleic acid. Commercially fried nuts should be

TABLE II

Basic Meal Plan (For Group I)

Breakfast:
- 1 cup 10% fruit
- 1/2 cup cooked whole grain cereal
- 1 tbsp oil added to cereal during cooking
- 1 cup skimmed milk
- Coffee or tea, as desired, with all meals

Lunch:
- Soup made with skimmed milk, vegetables and part of oil
- 1/3 cup low calorie cottage cheese
- Cooked or raw non-starchy vegetable, as desired
- 1/3 cup nuts
- 1 slice low-fat bread
- 1 1/2 tbsp oil added to soup or vegetable or as salad dressing. Make salad dressing using egg white, but no yolk, so that 1 tbsp dressing = 2 tsp oil
- 1/2 cup 20% fruit or 1 cup 10% fruit

Dinner:
- 1/3 cup low calorie cottage cheese
- 1 cup 20% vegetable
- Other vegetables, as desired, cooked or raw
- 1 1/2 tbsp oil, used as salad dressing, or added to vegetables
- 1/2 cup 20% fruit or 1 cup 10% fruit
- 1/3 cup nuts
- Complete skimmed milk allowance

Make meal plan changes according to each group food allowance.

TABLE III

2,000 Calorie "Safflower Milk" Diet

Fat: 120 g; Protein: 71 g; Carbohydrate: 167 g; Linoleic Acid: 85–90 g

Food (use daily)	Household measure	Gram weight	Protein	Fat	Carbohydrate
"Saf-Lac" (mix total solids in 7 cups water as day's supply)	3 1/2 cups	300	63	120	92
10% fruit	2 cups	400	—	—	40
7% vegetable	1 cup	200	4	—	14
Group A unclassified vegetables	As desired	—	4	—	15
Coffee or tea	As desired, all meals	—	—	—	—
	Total	—	71	120	161

All food should be prepared without added fat. "Saf-Lac" may be used in soups or as a vegetable sauce. Only noncaloric sweeteners should be used. The unclassified vegetables may be eaten in any reasonable amounts.

avoided, but nuts may be fried at home in one of the recommended oils.

Iron and Vitamin Supplements

All the diets described require iron and multi-vitamin supplementation.

Vegetable Fat "Cream"

A "cream" which may be used in coffee or with cereals and fruit may be prepared by homogenizing in a blender: 1/3 cup (80 ml) safflower, corn or cottonseed oil; 1/3 cup (40 g) nonfat milk solids; 1 1/2 cups (300 ml) water; and 1/2 to 1 teaspoon (1.5–3 g) of emulsifier (Gliddophil S.C., obtainable from Glidden Co., Chicago).

SPECIAL INSTRUCTIONS FOR GROUP II AND III DIETS

Group II

Liver, fish, and both dark and light meat of lean fowl are allowed. Remove skin and visible fat.

Group III

Lean meat includes lean beef round and sirloin, lamb leg, and lean ham. Allow three eggs per week in this diet. When an egg is taken, reduce cottage cheese to 1/4 cup.

Coffee and tea, sweetened with Succaryl® or saccharin, may be used as desired.

The amounts of linoleic acid indicated are based upon the use of safflower oil (approximately 75 per cent linoleic acid). If corn or cottonseed oil are used (approximately 50 per cent linoleic acid) the amounts must be increased and carbohydrate calories (bread and 20 per cent vegetables) decreased proportionately.

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Experimental Arteriosclerosis in Pyridoxine-Deficient Rhesus Monkeys

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For a number of years our laboratory has been engaged in the study of the biochemical and pathologic changes induced in monkeys by deficiencies of ascorbic acid and of various members of the B-complex. In collaboration with the late Dr. J. F. Rinehart, who was Professor of Pathology at the University of California School of Medicine, studies of pyridoxine deficiency were undertaken in 1945 with a small series of monkeys. In 1948[1] and 1949[2] we presented our initial observations on the occurrence of arteriosclerotic lesions in monkeys maintained on a diet deficient in vitamin B_6. In a subsequent report[3] we pointed out the essential similarity of the experimental vascular lesions of the monkey with those of arteriosclerosis of man.

The studies have been continued and to date we have examined approximately 50 animals which have been subjected to acute and chronic pyridoxine-deficiency and also a considerable number of animals which have been provided with complete supplements or deprived of other vitamins. The lesions have been observed consistently in monkeys exposed to complete deficiency of pyridoxine for six months or longer and also in monkeys maintained on suboptimal intakes of the vitamin for protracted periods of time. Confirmation of the occurrence of the vascular lesions in the vitamin B_6-deficient rhesus monkey and observations on the development of similar lesions in dogs maintained on a diet deficient in pyridoxine have been described by Mushett and Emerson.[4]

The experimental sclerotic lesions of the pyridoxine-deficient monkey are widely distributed and involve arteries of all sizes. They have been observed in the abdominal aorta, iliac and femoral arteries, in the arteries of the testicular tunic, and in the small and medium sized branches of the renal arteries. In a number of animals lesions have been observed in the coronary arteries, mesenteric arteries, and arteries of the pancreas and other viscera. For a detailed description and photographic examples of the lesions, the reader is referred to previous publications.[2,3,5] The vascular lesions resemble those observed in human arteriosclerosis in two essential points: (1) the distribution of the lesions follows the pattern found in man; and (2) the basic pathologic process appears to be similar.

Since hypertension is a factor which may contribute to the development of arteriosclerosis, it is important to know whether monkeys deprived of vitamin B_6 develop high blood pressure. For this reason measurements of the systolic blood pressure have been carried out in a number of control and pyridoxine-deficient monkeys. In eleven control and thirteen deficient animals the mean blood pressure with mean deviation and range are, respectively, 119 ± 10.7 (95–135 mm Hg); 120 ± 9.4 (105–130 mm Hg). There is no significant difference between the mean blood pressures of the two groups and hence hypertension would not be expected to play any role in the development of the vascular lesions in the vitamin B_6-deficient monkey. Olson and Martindale[6] reported an increase in systolic blood pressure of rats suffering from chronic vitamin B_6 deficiency. However, the experimental procedure used by these

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authors was not one of simple dietary restriction of the vitamin, since all their deficient rats had received the vitamin B_6 antagonist, desoxypyridoxine, at one time or another during the course of the experiment. Sinclair[7] has stated that he has never observed hypertension in pyridoxine-deficient rats in his laboratory.

It is always of interest to attempt to find fundamental chemical mechanisms which might explain the occurrence of gross or microscopic pathology in tissues. In the case of the vitamin B_6-deficient monkey there are at least two plausible explanations for the development of the arteriosclerotic lesions. The first of these is concerned with the formation of phosphatidyl choline (lecithins). Pilgeram[8] has published evidence in support of the thesis that in atherosclerosis there is a metabolic block in the formation of phosphatidyl choline. In order to explain the occurrence of vascular lesions in the pyridoxine-deprived macaque he has suggested that there might be a block in the decarboxylation of serine to form ethanolamine, a reaction presumably catalyzed by pyridoxal phosphate.[9,10] Such a block could conceivably interfere with the synthesis of phosphatidyl choline by limiting the supply of ethanolamine for the formation of choline.

A second possible mechanism is related to the role of vitamin B_6 in the metabolism of essential fatty acids. Schroeder[11] and also Sinclair[7] have speculated on the possibility of a deficiency of essential fatty acids being involved in the development of atherosclerosis. Sinclair has expressed the belief that a combined deficiency of vitamin B_6 and arachidonic acid may be "the reason for the occurrence of atherosclerosis in pyridoxine deficient monkeys." It is conceivable that vitamin B_6 deficiency might result in interference with the metabolism of essential fatty acids, since Witten and Holman[12] have presented evidence that vitamin B_6 is required by the rat for conversion of linoleic and linolenic acids to higher polyunsaturated fatty acids, namely, arachidonic, pentaenoic, and hexaenoic acids. At present, no pyridoxal phosphate catalyzed reaction is known which is applicable to this conversion.

Experiments designed to explore the relationship of essential fatty acid deficiency to the vascular disease have been in progress for approximately 20 months. Monkeys subjected to single and combined deficiencies of essential fatty acids and vitamin B_6 are being investigated. It is still too early to make any statement concerning the effect of essential fatty acid deficiency alone or in combination with pyridoxine deprivation on the course of development of the vascular lesions. It is sufficient to say that the growth of animals on the essential fatty acid-deficient diet is perhaps only slightly inferior to that of control monkeys and there is little, if any, change in their outward appearance or behavior. On the other hand, animals on the combined deficiency may fail somewhat earlier than monkeys subjected to simple vitamin B_6 deficiency.

SUMMARY

A brief review of the occurrence of arteriosclerotic lesions in the vitamin B_6-deficient monkey is presented. Measurements of the systolic blood pressure show that hypertension is absent in these monkeys. Some of the possible mechanisms relating to the development of the arteriosclerotic lesions are discussed.

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The Origins of Plasma Cholesterol

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THERE is a small positive correlation between the plasma cholesterol concentration and the development of clinical disease ascribed to atherosclerosis. This is true, however, only on a class or statistical basis. It is of no predictive value for individuals, whether "normal"[1] or diabetic.[2] Nevertheless the correlation justifies our studies concerning the origin and fate of plasma cholesterol.

Cholesterol enters the plasma either by absorption or by synthesis and release from the tissues.

EFFECT OF ABSORPTION

All exogenous cholesterol enters the blood by way of the thoracic lymph.[3,4] When cholesterol C-14 was given to a human by mouth, all radioactivity appeared in the lymph, none in the portal vein blood. Half of the lymph cholesterol C-14 was esterified, although all was given in the free form.[5] The newly absorbed cholesterol is not evenly distributed in the lymph but is preferentially concentrated in the chylomicrons (Fig. 1).[5,6,7]

The higher specific activity of the chylomicrons shows that exogenous cholesterol is probably built into them during passage from the intestinal mucosa to the lymph. The presence of these chylomicrons makes it possible to study the immediate fate of exogenous cholesterol separately from endogenous cholesterol.

After entering the blood, the chylomicrons leave again in a matter of minutes.[8] The major portion of the cholesterol enters the liver.[9,10] Once it has entered the liver, exogenous cholesterol is metabolized in the same way as endogenous cholesterol.[11] Absorbed cholesterol adds quantitatively to plasma cholesterol only during the persistence of alimentary lipemia. Its quantitative effect is slight and transient.[12]

EFFECT OF RELEASE FROM TISSUE (SYNTHESIS AND STORED EXOGENOUS CHOLESTEROL)

Almost all tissues synthesize cholesterol.[13] The newly synthesized cholesterol quickly appears in plasma.[10] The removal of various organs, however, does not alter plasma cholesterol responses, excepting only removal of the liver.[14,15,16] This holds true for hypercholesterolemia responses, such as bile duct ligation,[14] response to Triton® injection,[17] response to plasma albumin loss,[18] and also for restoration of normal plasma cholesterol values after acute reduction. If C-14 labeled acetate is given to a dog, C-14 labeled cholesterol is synthesized in almost all tissues, yet C-14 cholesterol appears in the plasma only if the liver is functioning.[15] The same observation has been made in heart-lung-liver preparations, using heart-lung preparations as controls.[16] Such data have justified the statement that the

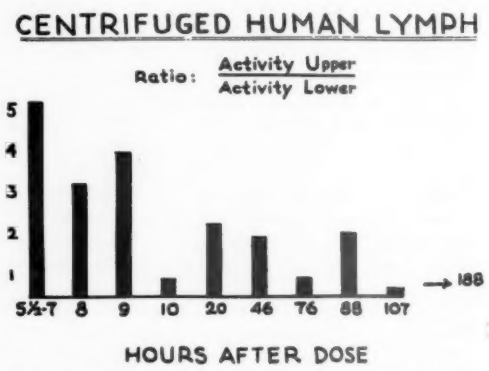

Fig. 1. Radioactivity from cholesterol-4-C^{14} absorbed into human intestinal lymph after oral administration. The upper, chylomicronous layer, shows preferential incorporation of radioactivity.

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liver is the chief contributor to plasma cholesterol.

However, the liver is not the only possible contributor, for under special circumstances a substantial rise in plasma cholesterol can be brought about in the hepatectomized rat. One such condition is massive continuous infusion of phospholipid.[19] This action is shown by a variety of phospholipids, (except perhaps duck egg yolk lecithin purified through the cadmium salt which appears not to be active). This effect of phospholipid apparently mediates the hypercholesterolemia consequent upon biliary obstruction.[20] The hypercholesterolemia consequent on biliary obstruction can be duplicated in the normal animal by phospholipid injection to simulate the phospholipid rise which follows biliary obstruction. Since such phospholipid injection does not raise blood bile acids, the phospholipid rise seems a more immediate cause of hypercholesterolemia than does bile acid.

Another material whose continuous massive injection will raise blood cholesterol is triglyceride fat. The action of triglycerides is not mediated by phospholipid, although phospholipid also increases after triglyceride injection.[21] The triglyceride must be given continuously, or it escapes from the plasma, even in the hepatectomized rat.[22] Triglyceride fat accumulation apparently mediates the hypercholesterolemia consequent upon Triton injection,[22–25]

Fig. 2. Appearance of aorta from an untreated atherosclerotic rabbit (#112, at left side) contrasted with that from a matched control rabbit treated with repeated infusions of phospholipid.

and also the hypercholesterolemia of nephrosis.[26] The action of phospholipid or triglyceride is to impede the exit of cholesterol from plasma to the liver, while not affecting its entrance into the plasma.[20–25]

Where does the cholesterol, which appears in the plasma after these agents, come from?

TABLE I

(A) Effect of Phosphatide Infusion on Aortic and Coronary Atherosclerosis

Number of rabbits	Average plasma cholesterol during cholesterol feeding		Average plasma cholesterol at beginning of experiment		Number of infusions	Gross aortic atherosclerosis (0–5)			Microscopic examination of left coronary artery (% occlusion)		
	Phosphatide infused	Controls	Phosphatide infused	Controls		Phosphatide infused	Controls	Difference (%)	Phosphatide infused	Controls	Difference (%)
7	981	972	131	138	8	2.7	4.9	82	35	58	81

(B) Effect of Phosphatide Infusion on Aortic Cholesterol and Lipid Contents of Atherosclerotic Rabbits

Number of rabbits	Aortic cholesterol (g/100 g)			Aortic total lipid (g/100 g)		
	Phosphatide infused	Controls	Difference (%)	Phosphatide infused	Controls	Difference (%)
7	15.2 (±1.8)	23.0 (±1.7) +<0.001	51	47.2 (±4.7)	64.6 (±5.1) +<0.01	37

Quantitative analyses have not been sufficiently accurate to assign its proper contribution to each tissue. One such tissue is the liver. But comparable plasma concentrations can be obtained in the absence of the liver. We believe the source to be a general contribution from many tissues. The adrenal is one where a quantitative deficit may be demonstrated.[23,27] In other tissues biologic and analytic variations have so far prevented a clear demonstration.

One other tissue which can be shown to lose cholesterol to plasma is the atherosclerotic aorta.[28] This has been shown by analysis following repeated injection of phospholipid into atherosclerotic rabbits. The effect of infusions repeated over three weeks is shown in Table I. Evaluation of aortal atherosclerosis at the end of this period is presented in Figure 2.

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The Mechanism for Changes in Blood Cholesterol in Deranged Thyroid States

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THE PLASMA cholesterol level rises in the hypothyroid state and falls in the hyperthyroid state,[1–7] although without a close or immediate relation with the basal metabolic rate.[8] Feeding excess cholesterol to animals will increase the hypercholesteremia of the hypothyroid state.[7,9] However, low-sterol diets are without effect[4] and changes in intestinal absorption of cholesterol do not account for the blood cholesterol changes in either hypo- or hyperthyroid states.[5,10,11] No redistribution of cholesterol between the blood and fixed tissues occurs which might explain the change in blood levels.[12,13]

The rate of synthesis of lipids and phospholipids is apparently increased in hyperthyroidism.[14–19] This is true also of cholesterol synthesis.[12,17,22] Conversely, there is a lower synthesis rate in hypothyroidism.[12,17,21,22]

These changes in rate of synthesis are of course opposite in direction to the blood cholesterol concentration changes. In order for the blood levels to vary as they in fact do vary, the excretory or destructive processes concerned with cholesterol must dominate in the hyperthyroid state and be subordinate to synthesis in the hypothyroid.

Where a radioactive label is incorporated from body water into cholesterol and the rate of loss of labeled cholesterol observed, it is noted that cholesterol is lost more rapidly in hyperthyroid rats than in normal rats, while loss of cholesterol is diminished in hypothyroid animals.[12] Here is a rate process in the same direction as the blood change.

TABLE I

Rate of Disappearance of Injected Cholesterol in Hypo- and Hyperthyroid Rats*

Type of rat	Number of rats	Plasma cholesterol increments (mg/100 ml)†				
		Immediately after injection	1 hr after injection	3 hr after injection	6 hr after injection	12 hr after injection
Control	5	114 ± 5.4	113‡	69.6 ± 3.4	33 ± 3.5	9 ± 3.5
Hyperthyroid	7	137 ± 7	103 ± 6.5	58.4 ± 8	6 ± 4.1	−18 ± 7.4
Hypothyroid	6	127.6 ± 8.4	118 ± 3.4	90 ± 6.3	51.5 ± 9	10.8 ± 4.4

The figures following the ± sign refer to the standard error of the mean.

* Hypercholesteremia was induced by intravenous injection of hypercholesteremic rat plasma.

† These increments represent the plasma cholesterol (mg/100 cc) in *excess* of the pre-injection values.

‡ Number of rats too small to permit calculation of the standard error.

The rate of disappearance of injected physiologic cholesterol is in keeping with this view (Table I).[12,20]

The rate of excretion of sterols is greater in hyperthyroid animals than normals and is less in hypothyroid animals,[12] even though all the animals eat a sterol-free diet. Thus a disparity between rates of synthesis and destruction or excretion underlies the inverse thyroid activity/blood cholesterol relationship.

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Plant Sterols and Tissue Cholesterol Levels

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AMONG the many dietary substances known to affect the level of cholesterol in blood and other tissues are the plant sterols.[1] Much of the data on this subject, which have been interpreted as being in conflict, have been obtained with considerable variation in experimental conditions. Soybean sterols or sitosterols fed to chickens in a cholesterol-enriched diet will prevent the usual elevation of plasma cholesterol which occurs on such a diet.[2–7] Liver deposition of cholesterol is also reduced and the occurrence of atheromatous plaques due to prolonged cholesterol feeding is significantly reduced.[3,4] Similar effects have been obtained with β-sitosterol, stigmasterol and ergosterol.[8,10] The effects of dihydrocholesterol, similar on short-term feeding,[9] are found to differ on prolonged administration.[48] Estrogen-induced hypercholesteremia in the chicken is not prevented by plant sterols.[5]

Prevention of hypercholesteremia[11,12] and of atherosclerosis[11] in cholesterol-fed rabbits has been obtained by feeding soy sterols, tall-oil sterols and β-sitosterol. Higher ratios of plant sterol to cholesterol (4:1–7:1) appear to be required in rabbits than in chickens since a 1:1 ratio was ineffective in rabbits.[13]

Swell *et al.*[14] found that feeding of soy sterols to rats, on a 2 per cent cholesterol diet, inhibited the increase in *whole blood* cholesterol. Rosenman and co-workers[15] and Ahlfin-Slater *et al.*[16] found no effect of dietary soy sterols on *plasma* cholesterol in the cholesterol-fed rat. No change occurred in total carcass cholesterol[16] but liver lipids and liver total cholesterol were much lower than in cholesterol-fed rats.[16,18,19] Deposition of fed cholesterol-C^{14} in rat livers was decreased by feeding soy sterols.[17] (It should be noted that the normal rat does not respond to cholesterol feeding with the enormous hypercholesteremia seen in the chicken and the rabbit.)

Human Studies: Reduction of blood cholesterol in man by feeding soy sterols was first reported by Pollak.[20] Many others have since reported reduction in serum cholesterol by feeding plant sterols. Data from five of these studies on 66 patients[21–25] indicate that about one-half showed reductions in serum cholesterol of less than 15 per cent and the other half greater than 15 per cent. In most cases a commercial suspension of sitosterols has been used, the composition of which other than sterols has not been stated. Some have used sitosterol in biscuit form.[26] Doses have varied widely from 9 to 25 g or greater, per day, consumed before meals. Serum cholesterol rose on cessation of sterol feeding but usually there has been no spontaneous rise during experimental periods even when continued as long as 18 months.[24]

Wilkinson and associates,[27] Friedman *et al.*[28] and Pomeranze and Chessin[22] have reported no effect of sitosterols on serum cholesterol, although the latter group have fed smaller amounts than others. In some hypercholesteremic individuals the fluctuations in serum cholesterol are greater than those obtained during periods of sitosterol therapy.[30] Similar observations have been made on monkeys.[31]

It is interesting that although most of the animal experimenters have mixed the plant sterols with dietary fat, this has been done in only a few cases in human studies. The effectiveness of plant sterols in reducing serum cholesterol appears to be greatly increased by incorporation into dietary fat. Peterson *et al.*[32] fed 5.7 g of soy sterols per day incorporated

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into butter consumed with meals and obtained reductions of up to 20 per cent in plasma cholesterol (mean 11 per cent), as great as most workers have obtained with three to four times this amount. Beveridge, Connell, and Mayer, using 7 g β-sitosterol per 950 calories in a homogeneous formula diet, obtained reductions of over 20 per cent in seven out of nine subjects.[33] Farquhar and Sokolow[35] obtained a 20 per cent reduction in β-lipoprotein cholesterol with either 81 g per day of safflower oil or 18 g of sitosterol. When both were fed, the fall was 32 per cent (mean of 15 patients). The effectiveness of plant sterols employed in this manner have led some[33] to suggest that sitosterol may be the cholesterol-depressing factor in corn oil. It would appear that the levels used were much higher than those present in corn oil, however, although these same workers have recently reported that much lower levels of sitosterol are also effective.[34]

ABSORPTION AND METABOLISM OF PLANT STEROLS

Using tritium-labeled β-sitosterol, Gould and associates[36] showed, in single dose feedings (10 mg), that it was absorbed by rats about one-fifth to one-tenth as completely as an equal dose of labeled cholesterol. The absorption rate on continuous feeding was about one-tenth that of cholesterol. β-sitosterol feeding did not depress cholesterol synthesis from acetate in rat liver or intestine.[37] Labeled β-sitosterol replaced about 7 per cent of the liver cholesterol in rats as compared with 49 per cent for labeled cholesterol and 3.5 per cent of carcass cholesterol compared with 26 per cent for cholesterol. In contrast to this the replacement of cholesterol by dihydrocholesterol was much greater.[36]

In five human subjects fed labeled β-sitosterol, the absorption rate, as judged by plasma sterol level, appeared to be about one-tenth that of labeled cholesterol. Gould suggests that the metabolism of β-sitosterol is different from that of cholesterol since the specific activity curves for red blood cell, plasma free, and plasma ester cholesterol did not approach equality as when labeled cholesterol is fed.[37] In one study on a terminal carcinoma patient 29 days after feeding, the highest sterol specific activity was found in the bile as compared with whole blood and liver. The lowest activity was found in a section of aorta containing plaques.[37]

Studies using the balance technic with rats showed that when soy sterols were fed from 16 to 26 per cent disappeared from the intestine, indicating absorption or destruction.[38,39] Soy sterols tended to increase the sterol content of the serum. When both cholesterol and soy sterols were fed, the sterol elimination was greater than the sum of the sterol elimination when either was fed alone.[39] More recently, using tritium-labeled β-sitosterol, Swell and associates[40] found that β-sitosterol increased the excretion of other sterols although more than half of the β-sitosterol itself disappeared from the intestinal tract. In spite of this, soy sterols tended to decrease the liver sterol content as compared with the same diet lacking soy sterols.[38] Swell and associates were unable to demonstrate the presence of soy sterols in either liver or plasma by chromatography, indicating that they represented less than 5 per cent of the total sterols.[38] Using an x-ray diffraction technic Shipley *et al.*[41] were unable to demonstrate β-sitosterol in livers of rabbits which had been fed β-sitosterol (4 g daily) for periods of over two years indicating that less than 2 per cent of the total sterols was β-sitosterol.

Some question may be raised as to whether or not balance studies on digitonin-precipitable sterols[38,39] are adequate to determine absorption since the possibility exists that conversion to non-β-sterol substances by intestinal bacteria may occur.[42,43]

One study on C^{14}-labeled ergosterol[44] showed that 2 to 5 per cent of 1–2 mg doses were absorbed by rats. Most of the radioactivity was excreted in the bile and the absorbed ergosterol was not converted to cholesterol.

It is apparent that little is known concerning the metabolism of plant sterols. Adequate analytical methods still remain to be developed for their detection and differentiation from other sterols.

Toxicity Studies

Because some misunderstandings have arisen

it is desirable to point out that dihydrocholesterol does *not* occur among plant sterols and is a normal constituent of animal tissues. Although feeding of dihydrocholesterol in a cholesterol-enriched diet results in an inhibition of the increases in plasma cholesterol in chickens,[9,10] rabbits,[46] and rats,[47] it has been found that prolonged feeding (six months) of 2 per cent dihydrocholesterol to chickens produced severe arteriosclerosis and enlargement and lipid infiltration of the liver.[48] Rabbits fed 2 per cent dihydrocholesterol for seven months developed extensive plaque formation in the aorta and cirrhosis of the liver.[49] Increased β-sterols and cholesterol were found in livers and aortas.

On the other hand rabbits fed 2 per cent β-sitosterol for seven months developed no changes in liver or aorta or in total β-sterols of these tissues.[49] Feeding of both cholesterol and soy sterols to chickens for 28 weeks resulted in a degree of atherosclerosis that was no greater than that of control (non-sterol-fed) birds.[3]

The report of Curran and Costello[50] that the feeding of soy sterols to four rabbits for four weeks produced atherosclerosis and deposition of soy sterols in the aortas has not been confirmed by others[41,49] who have fed plant sterols for much longer periods. No description of the two control rabbits in Curran and Costello's experiment was given nor of the infrared technic used for determining the soy sterols. Shipley *et al.*[41] have fed very high levels of sitosterols to rabbits, rats, and dogs for periods up to two years without development of pathologic alterations of tissue or increase in sterol content.

Mechanism of the Effect of Plant Sterols

Soy sterols and β-sitosterol apparently do not effect decreased tissue levels of cholesterol by inhibiting its synthesis from acetate.[37,51] There is no effect on estrogen-induced lipemia in the chickens.[5] Nevertheless, plant sterols *have* been shown to decrease absorption of C^{14}-labeled cholesterol in rats[52,53] as measured by recovery of the labeled compound in thoracic duct lymph. Similar effects have been found with dihydrocholesterol and ergosterol.[54] Recovery of labeled cholesterol in livers of rats fed soy sterols was also decreased.[17] The hypocholesteremic effects produced in man may possibly be related to interference with reabsorption of cholesterol since dietary cholesterol appears to have little effect on blood cholesterol in man.[45]

Two mechanisms have been suggested for this effect: (1) that plant sterols form insoluble mixed crystals with cholesterol, decreasing its absorption;[11,55] (2) that the plant sterols compete with cholesterol for the absorption mechanism.[8,39,52,56] No evidence from animals has been presented for the first. The evidence for the second is indirect. The suggestion has been made that plant sterols may compete in a cholesterol esterification system involved in absorption[8,52,56] since cholesterol is esterified in the absorption process.[59,66] Soy sterols, ergosterol, and dihydrocholesterol fed as esters have diminished capacity to prevent increases in plasma and liver cholesterol in chickens.[8,10] Esterified β-sitosterol similarly has little effect on liver cholesterol in rats.[18,19]

Cholesterol, itself, fed as the ester, has diminished or lost completely the ability to increase plasma and liver cholesterol in the chicken[10] and in the rat.[57,58] The absorbability of the ester has been related to its rate of hydrolysis by pancreatic cholesterol esterase.[57] Evidently only *free* cholesterol is absorbed, and all ester cholesterol is *not* hydrolyzed in the intestinal tract. The esterification which occurs during absorption does not take place in the intestinal lumen, since the mucosa contains mostly free cholesterol[59] and cholesterol ester is less readily absorbed than free cholesterol. It has not been established that pancreatic cholesterol esterase is the esterifying enzyme involved in absorption, although it has been reported that exclusion of pancreatic juice decreases the content of esterifying enzyme in mucosa.[60] Although Hernandez *et al.* found that exclusion of pancreatic juice decreased cholesterol absorption in the rat[61] the results of Byers and Friedman[62] and Lin *et al.*[63] are in disagreement with this. Lin *et al.* suggest that the effect of pancreatic juice is to provide free fatty acid (needed for cholesterol absorption), a factor not provided for in Hernandez' experiments. Pancreatectomy does not reduce

alimentary hypercholesteremia in the chicken.[64,65] Furthermore, absorbed cholesterol bears a constant ratio of ester to free cholesterol regardless of interference with absorption by other sterols,[54] suggesting that the plant sterol effect occurs at another level than that of the esterification occurring during absorption. Since it has been found by Swell *et al.*[14,56] that plant sterols are more slowly esterified by pancreatic cholesterol esterase than cholesterol itself, the possibility is suggested that the presence of plant sterols may in some manner favor the esterification of cholesterol in the intestinal tract, which as seen from the above discussion would tend to decrease cholesterol absorption.

Since it now appears that lowering of serum cholesterol may be accomplished by nutritional means it is probable that the use of plant sterols for this purpose may have but limited pharmacologic applications. However, further study of the mechanism of action of plant sterols and of their metabolism would appear to be valuable in elucidating the mechanisms involved in the absorption of cholesterol itself.

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The Essential Fatty Acids

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IN SOME studies of the fatty acid composition of lipids from human blood,[1] it was found that the composition of the human erythrocyte was remarkable for its constancy. This fact prompted an investigation of lipid synthesis by the erythrocyte by incubating sterile whole blood with CH_3-labeled acetate.[2] After a 5-hour incubation not only the red cell lipids but also the plasma lipids were found to be labeled.

Separation of the long chain fatty acids on the gas-liquid chromatogram followed by collection of the fractions and determination of their specific activity, demonstrated that labeling existed not only in all the common saturated and unsaturated acids but also in linoleic and arachidonic acids.

TABLE I

Specific Activities of Long Chain Fatty Acids Isolated from Red Cell and Plasma Lipids after Incubation with Labeled Acetate (counts/min/mg)

Acid	Red cell neutral fat	Plasma neutral fat	Red cell phospholipid	Plasma phospholipid
Lauric	325	1030	255	760
Myristic	660	520	506	612
Palmitic	132	180	41.0	570
Stearic	203	312	87.0	122
Linoleic + linolenic	—	61.0	34.0	31.0
Oleic	60	377	37.0	40.0
Arachidonic	Not determined	Not determined	18.1	207

In Table I are shown the specific activities of a variety of fatty acids isolated from the lipids of whole blood in a typical experiment.

The fatty acids synthesized by the red cell are incorporated into neutral fat and phospholipid which are exchanged with the plasma lipoproteins.

The linoleic and linolenic acids in the plasma neutral fat have a specific activity of one-fifth of that of oleic acid and may therefore represent a turnover of the same order. It is of some interest to note that in the original experiments of Stetten and Schoenheimer on the use of tracers in the biosynthesis of fatty acids the isolated linoleic acid possessed 0.02 atom per cent excess of deuterium compared with 0.06 for oleic acid.

The presence of radioactive label in the linoleic plus linolenic acids isolated from whole blood does not furnish proof of their biosynthesis since two possibilities exist:

(1) An exchange process such as:

$$\text{linoleic acid} \rightleftharpoons \text{degradation product} + \text{acetate} \quad \text{acetate} \rightleftharpoons \text{acetate pool}$$

(2) Synthesis from acetate or from some unessential fatty acid and acetate.

The small amounts of material so far available have prevented a stepwise degradation that would accurately define the position of the label. Those experiments carried out so far have shown part of the activity to exist in the azelaic acid obtained by oxidative degradation.

Nevertheless, this synthetic activity of the red cell, when taken together with the exchange of lipids with plasma lipoproteins, does raise the question of the importance of dietary linoleic and arachidonic acids for the human. The precise requirements, if any, of these acids for the adult human has yet to be defined. In recent years many suggestions have been made, particularly by Sinclair,[4,5] that some human diseases were caused by dietary insufficiency of the essential fatty acids. Such insufficiency would be expected to be detectable in the blood

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levels of humans suffering from these diseases since Mukherjee *et al.*[6] demonstrated a marked fall in di- and tetraenoic acids in the plasma of rats when fed a fat-deficient diet.

Such studies as have been made on (a) adults suffering from coronary artery disease[7] and (b) children suffering from idiopathic hypercalcemia[8] have failed to demonstrate significantly lower blood levels of linoleic plus linolenic and arachidonic acids than those found in control subjects.

Since neither the detailed function nor the required level of intake of these unsaturated fatty acids is understood, at this stage it would be useful to devote less time to sermonizing and more time to experimentation on the subject of essential fatty acids.

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Interconversions of the Saturated and Monounsaturated Fatty Acids

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BEFORE the advent of radioactive or isotopic tracers in the study of fatty acid metabolism, considerable evidence was available which indicated that the fatty acid composition of the body fats of mammals is determined in the following ways:

(1) By direct deposition of dietary fat.

(2) By synthesis from carbohydrate or other sources.

(3) By alteration of fatty acids derived by the first two methods. It is with the third method that the present discussion is concerned.

The work of Schoenheimer and his collaborators, using deuterium, laid the groundwork for all the more recent studies of fatty acid interconversions. After feeding biosynthetically derived fatty acids containing deuterium, Schoenheimer and Rittenberg[1] found that the unsaturated fatty acids of the rats contained appreciable amounts of deuterium (one-fifth to one-half the amount of the saturated fatty acids isolated from the same rats). When biosynthetically prepared unsaturated fatty acids were fed, the saturated fatty acids contained one-fifth to one-fourth the deuterium found in the unsaturated fatty acids.[2] These amounts were the same as the deuterium content of the body water of the two rats. Since when water is the only source of deuterium, the saturated fatty acids of rats reach a maximum content about one-third that of the body water, Rittenberg and Schoenheimer concluded that at least two-thirds of the deuterium of the saturated fatty acids was due to hydrogenation of the fed unsaturated acids. It is possible, however, that this conclusion is not justified, since in the experiments under consideration, the deuterium content of the saturated fatty acids could have been derived both from body water and from the break-down products of the unsaturated fatty acids.

In a further study, Stetten and Schoenheimer[3] found that deuteropalmitic acid was converted partially into stearic and palmitoleic acids. Bernhard and Schoenheimer,[4] on the other hand, found that linoleic acid obtained from rats maintained on a high-deuterium water contained little or no deuterium despite the fact that the other fatty acids showed appreciable amounts.

The results of three studies were summarized by Schoenheimer as follows:

$$\begin{array}{ccccc} \text{Stearic} & \rightleftharpoons & \text{Palmitic} & \rightarrow & \text{Shorter chain} \\ \downarrow\uparrow & & \downarrow\uparrow & & \\ \text{Oleic} & & \text{Palmitoleic} & & \end{array}$$

The use of C^{14} permitting the labeling of specific carbon atoms provided more definitive evidence on the interconversions of the saturated acids. Weinman and his co-workers[5] first administered palmitic acid (as tripalmitate), containing C^{14} in the first or sixth carbons, to rats and monitored the rate of elimination of $C^{14}O_2$. Since no difference could be detected by this means in the rates of oxidation of the different types of palmitic acid, it was concluded that once oxidation of palmitic acid was initiated, it proceeded to completion with very little stabilization of shorter chain acids, such as myristic or lauric, taking place. When stearic acid labeled in the same manner was administered intravenously to rats as tristearin emulsion, somewhat different results were obtained.[6] In this case, the stearic acid labeled

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in the 6 position was oxidized more slowly than that labeled in the carboxy group. Moreover, isolation of the palmitic and stearic acids from the rats revealed that the palmitic acid from the rats receiving the 6-labeled stearic acid was indeed active, although only one-twelfth as active as the C_{18} acids from the same animals. Thus it appears that in the oxidation of stearic acid, appreciable numbers of molecules escape at the C_{16} stage and mix with the body pools of palmitic acid.

The reverse reaction, conversion of palmitic to stearic acid, has also been shown to occur. In experiments concerning the mechanism of carbohydrate sparing of fatty acid oxidation, Lossow and Chaikoff[7] found that although most of injected palmitic acid was stored as such, some appeared in the C_{18} acids, especially in fed as compared with fasted rats. Since the carboxy carbon of the stearic acid contained only 2 per cent of the activity, it appeared that the activity was incorporated largely as palmitic acid rather than by total synthesis from acetate. These conclusions had been reached previously by others. Zabin,[8] using rat liver slices or intact rats, studied the incorporation of carboxy-labeled acetate into the saturated fatty acids of the livers. The activity of the carboxy group and of the last two carbons of the saturated fatty acids was checked in palmitic and stearic acids separated by distillation of the methyl esters. In the case of the fed animal, it was found that the activity from the acetate was distributed evenly throughout the palmitic acid chain. In the fasted animal or in liver slice experiments, however, the carboxy groups were several times more active than the average of the remaining carbons. Since most of the activity in the stearic acid was found in the carboxy group, it again appeared that this acid was formed largely by elongation of palmitic acid. This hypothesis has been confirmed by Lipsky, Haavik, Hopper and McDivitt,[9] who found that in the plasma of patients injected with acetate-1-C^{14} the peak activity appeared first in the palmitic acid and was followed by the peak in stearic acid.

That palmitic and higher acids can be formed from the lower acids has been shown by Anker.[10] Rats on a fat-free diet were fed 35 mg per day of myristic acid-1-C^{14} for three days and were then killed for extraction of total fatty acids. These were separated into saturated and unsaturated fractions by the lead salt technic. The methyl esters of the saturated and the hydrogenated unsaturated acids were fractionally distilled and were degraded stepwise by the Borodin (silver salt bromination) reaction. In the case of palmitic acid, the degradation was carried out for three steps and the last two carbons were also isolated as acetic acid by chromic acid oxidation. The results obtained can be represented as follows:

CH_3—	CH_3—	$(CH_2)_{11}$—	CH_2—	CH_2————	———COOH
0.5	1.5	23	54–59	1.4	10–14.5% of activity

Since the third carbon is considerably more active than any of the others, it is evident that it is derived from the carboxy group of myristic acid. The remainder of the chain is active by virtue of total synthesis of palmitic acid from diluted active acetate. It is noteworthy first, that the last two carbon atoms may not be as active as the average of the last 13, and second that there is considerable activity in the even carbon atoms. This latter activity may very well be an artifact due to overoxidation during the degradation procedure.

From the unsaturated acids were obtained, after hydrogenation, myristic acid (evidently from myristoleic) with most of the activity in the carboxy group, and stearic acid (evidently from oleic and linoleic acids) with 7–10 per cent of the total isotope in the carboxy group. From these data, it appears that carbon atoms 5 to 18 of the oleic acid were derived from myristic acid to which two acetate residues were added. As will be seen below, it is most likely that the oleic acid was formed by dehydrogenation of stearic acid containing this distribution of isotope.

The distribution of activity in fatty acids synthesized from 1-C^{14}-acetate by the intact mouse was investigated by Dauben, Hoerger and Petersen.[11] Four hours after subcutaneous injection of the active sodium acetate, the mice were killed and the fatty acids were isolated from the saponified carcasses and separated by fractional distillation of their methyl esters. The unsaturated fraction separated by the lead salt technic, was hydrogenated prior to distilla-

tion. Each fatty acid was then degraded stepwise, and the activity of the first three or four carbon atoms was assayed. In some cases, the last two carbons were also obtained for counting by oxidation with chromic oxide. Following are the results obtained, expressed as per cent of total activity for each carbon atom or group of carbon atoms:

Saturated Acids

C_{16}	$CH_3—CH_2—(CH_2—CH_2)_5—CH_2—CH_2—CH_2—COOH$					
	66		2	12	1	13
C_{18}	$CH_3—CH_2—(CH_2—CH_2)_6—CH_2—CH_2—CH_2—COOH$					
	75		1	8	0.5	20

Unsaturated Acids

C_{16}	$CH_3—CH_2—(CH_2—CH_2)_5—CH_2—CH_2—CH_2—COOH$					
	76			14	1	15
	4					
C_{18}	$CH_3—CH_2—(CH_2—CH_2)_6—CH_2—CH_2—CH_2—COOH$					
	67			10	2	23

These results confirm the previous evidence that palmitic acid is totally synthesized from acetate, since the first and third carbons have approximately the same activity. However, the carboxy group of the stearic acid is approximately three times as active as the third carbon, confirming the hypothesis that stearic acid is synthesized to a great extent by elongation of palmitic acid.

In these experiments, as in those of Anker,[10] the distribution of activity in the unsaturated fatty acids closely corresponded to that of the saturated acids of the same chain length. This, together with their lower total activity would indicate that the unsaturated fatty acids are formed from the corresponding saturated acids by direct dehydrogenation. These ideas are supported by the experiments of Lipsky and his co-workers,[9] who found that in the serum of patients injected with radioactive acetate, the peak of activity of oleic acid followed that of stearic acid. Mead and Howton[12] also found that after the feeding of γ-linolenic acid (6,9,12-octadecatrienoic acid) to rats, the oleic and palmitoleic acids isolated from their organs and depot fat after four hours had one-seventh and one-fourth the activity respectively of the corresponding saturated acids. The indication, then, is that the saturated fatty acids are not formed by hydrogenation of the unsaturated acids, but that the reverse reaction is the normal mechanism for production of the monounsaturated fatty acids.

This probability makes likely the modification (Fig. 1) of Schoenheimer's original scheme for interconversion.

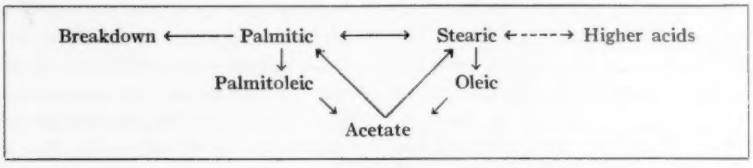

Fig. 1

Oxidation of the residual fatty acid from stepwise degradation of the 18-carbon unsaturated acids revealed only 4 per cent of the total activity in the last two carbons rather than a calculated 9 per cent. This is in agreement with the results of Anker[10] and may indicate that a process other than direct synthesis of palmitic acid from acetate can occur. As will be seen below, it is possible that formed acids can be partially degraded to all stages and rebuilt with active acetate. This would result in a slightly decreasing activity in the odd carbon atoms proceeding toward the terminal methyl groups. This process, then, would modify the more usual total synthesis and degradation of the 16-carbon acids.

SUMMARY

It appears that palmitic acid is largely synthesized or broken down with little formation of intermediate chain-length acids, but that this process can and does occur to a slight extent. Stearic acid is synthesized largely by elongation of palmitic acid and is partially degraded by removal of 2 carbons to form this acid. Palmitic and stearic acids can be dehydrogenated to the monounsaturated acids, palmitoleic and oleic, which are probably largely formed by this process. The reverse reaction, hydrogenation of the unsaturated to the saturated acids, however, probably does not appear to occur to any extent.

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The Metabolism of the Essential Fatty Acids

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PRIOR to the advent of isotopic tracers, evidence on the metabolism of the polyunsaturated fatty acids was obtained by alkali-isomerization analysis of tissue fats following the feeding of various fatty acids. This method, while it gave only indirect evidence, still furnished some interesting information. For instance, the feeding of a dienoic acid (linoleic) resulted in increases in dienoic and tetraenoic acids in the animal's tissues.[1,2,3] Feeding of trienoic acid (linolenic) resulted in increases in trienoic, pentaenoic and hexaenoic acids.[1,2,3] When animals were placed on a fat-free diet, increases in a trienoic acid were noted.[1,4,5] Other types of experiments gave additional information on the metabolic transformations of the essential fatty acids. For example, Bernhard and Schoenheimer[6] found that linoleic acid does not incorporate deuterium from the body water of animals although the saturated fatty acids and oleic acid incorporate fairly large amounts. Thus, the available evidence has indicated, first, that the essential fatty acids are not readily synthesized in the animal body (which could already be surmised from their nature) and second, that when obtained from an outside source, they can undergo various transformations.

The availability of fatty acids labeled with C^{14} in the carboxy group[7,8] and of methods for the separation and degradation of these fatty acids[9,10] has enabled many of the earlier results to be explained and the metabolism of the polyunsaturated fatty acids to be elucidated.

Acids were fed as methyl esters to rats on a fat-free diet. After four hours, the rats were killed, and their organ and abdominal depot fat was hydrolyzed to obtain the fatty acids. These were crystallized to separate saturated and unsaturated fractions, and the polyunsaturated fatty acids were isolated, as the insoluble polybromides, or by chromatography on the reversed phase chromatogram of Howard and Martin.[9] Fatty acids separated in this manner were degraded stepwise,[10] and the location of the C^{14} in the carbon chain was ascertained, where possible.

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LINOLEIC ACID

In the first experiment,[11] carboxy-labeled acetate was injected intraperitoneally. Linoleic acid isolated from these rats as the tetrabromide had a small amount of activity which, however, was almost completely removed by eight recrystallizations. The arachidonic acid from this experiment had essentially all its activity in the carboxy carbon. These results confirmed previous ideas that linoleic acid is not formed to an appreciable extent in the animal body and indicated that arachidonic acid is synthesized by addition of acetate to an 18-carbon exogenous precursor, presumably linoleic acid.

When carboxy-labeled linoleic acid was fed, active linoleic, arachidonic and probably docosapentaenoic acids were isolated.[12] Degradation of the arachidonic acid gave the following results, expressed as per cent of the total activity in each carbon atom:

$$\underset{0}{C_{17}H_{35}}\text{—}\underset{24.5}{CH_2}\text{—}\underset{0.7}{CH_2}\text{—}\underset{74.7}{COOH}$$

The complete absence of activity beyond the third carbon atom confirmed the hypothesis that the linoleic acid molecule is incorporated unchanged into arachidonic acid. The activity in the third carbon atom is undoubtedly that of the carboxy carbon of the linoleic acid. The surprisingly high activity in the carboxy car-

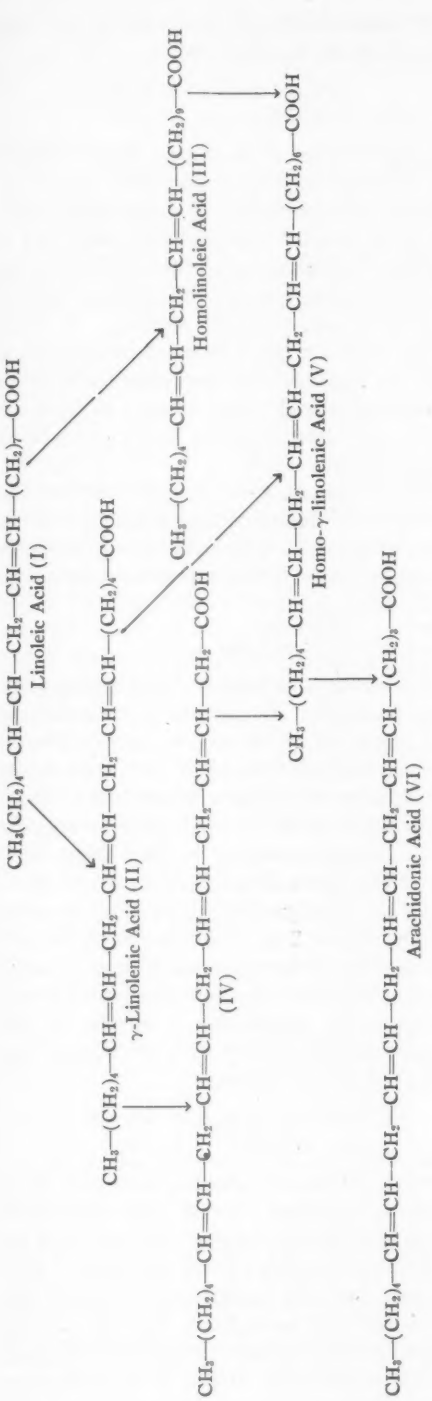

Fig. 1. Possible intermediate compounds in the transformation of arachidonic acid from linoleic acid.

bon undoubtedly comes from acetate formed by degradation of the active linoleic acid. In previous experiments[13] it was shown that fed linoleic acid is very rapidly oxidized. If the active acetic acid thus formed did not immediately mix with the general acetate pool but contributed to the chain-lengthening process without much dilution, a reasonable explanation for the relatively high activity of the carboxy carbon of arachidonic acid is on hand.

While these experiments demonstrated that arachidonic acid is formed in the animal body by addition of acetate to the carboxy end of linoleic acid, they did not indicate the order of events in this 3-step process involving one chain lengthening and two dehydrogenations. The possible intermediate compounds in this transformation are given in Figure 1. However, some evidence was available which aided in determining the most probable first step of the process. Thus, homolinoleic acid was reported by Karrer and Koenig[14] to be devoid of essential fatty acid activity and by Thomasson[15] to possess about 40 per cent of the activity of linoleic acid. Moreover, Thomasson reported that γ-linolenic acid was fully as active as linoleic acid. Hence, inasmuch as an intermediate in such a conversion should possess at least the activity of its immediate precursor, the pathway from I to II was definitely favored over that from I to III.

For these reasons, carboxy-labeled γ-linolenic (6,9,12-octadecatrienoic) acid was prepared from the seeds of *Oenothera Lamarckiana* and fed to rats in the usual manner.[16] In this case, as can be seen in Table I, almost all the activity in the fatty acids was found in the arachidonic acid. Several other interesting points are to be seen in the table. First, the linoleic acid from fraction F-3 (acetone-soluble at $-60°$) was active. However, linoleic acid isolated as the tetrabromide from fraction F-2 (acetone-insoluble at $-60°$, soluble at $-20°$) contained little activity. The activity in that from F-3, therefore, must have been due to a contaminant of similar chromatographic behavior, presumably the fed γ-linolenic acid. If this is true, only trace amounts of the fed acid were present four hours after feeding (its activity was 1.4×10^5 disintegrations per second

TABLE I

Weights and Activities of Lipid Fractions from 1-C^{14}-γ-Linolenate-fed Rats

	Weight g	Specific activity *d.p.s.* per mg*
Total fat	16.5	(9.5)
Total fatty acids	16.25	41
Fatty acid fraction I (acetone-insoluble at −20°)	4.95	15.2
Fatty acid fraction II (acetone-insoluble at −60°)	8.55	5.6
Fatty acid fraction III (acetone-soluble at −60°)	2.25	235

* d.p.s.—disintegrations per second.

per mg), again indicating almost complete conversion of the fed acid to arachidonic.

A second source of interest (Table II) is in the relative activities of the fed acid, the linoleic acid, the monounsaturated and the saturated acids. From these data, it does not appear that hydrogenation of polyunsaturated acids through the monounsaturated to the unsaturated acids is an important pathway if, indeed, it occurs at all. Finally, it can be seen that the second most active of the biosynthesized acids is a docosapentaenoic or docosahexaenoic acid presumably formed by addition of acetate to arachidonic acid. The distribution of activity

TABLE II

Activities of Fatty Acids from γ-Linolenate-fed Rats

Fraction	Component	Specific activity *d.p.s.* per mg*
F-1	Palmitic acid	1.9
	Stearic acid	2.5
F-2	Oleic acid	0.37
	Linoleic acid	0.63
F-3	Palmitoleic acid	0.45
	Linoleic acid (contaminated by γ-linolenic)	50.7
	Arachidonic acid	785
	Docosapentaenoic or docosahexaenoic acid	14.9

* d.p.s.—disintegrations per second.

in the arachidonic acid expressed as per cent of total activity is as follows:

$$\underset{1.2}{C_{17}H_{35}}—\underset{97.4}{CH_2}—\underset{0.3}{CH_2}—\underset{1.1}{COOH}$$

Once again it can be seen that the γ-linolenic acid molecule must have been used almost exclusively for formation of arachidonic acid. The small amount oxidized to acetic acid is shown by the low activity of the carboxy group as well as by the low activity of the saturated fatty acids from the same animals. It might be said, in this respect, that γ-linolenic acid is about the equivalent of arachidonic acid as an essential fatty acid. The activity beyond the third carbon atom deserves notice and will be discussed below.

Thus it appears at the present time that the pathway from linoleic to arachidonic acid is as shown in Figure 2. The last step in the process is under consideration in our laboratory at the present time.

LINOLENIC ACID

It is evident that linolenic acid has no place in the formation of arachidonic from linoleic acid. Experiments in which carboxy-labeled linolenic acid was fed to rats gave some information on the metabolism of this acid.[17] Both the C_{20} acid and the C_{22} acid (docosapentaenoate or docosahexaenoate) isolated from these animals were quite active. Linoleic acid, purified by chromatography of the tetrabromide, was only about 0.3 per cent as active as the arachidonic acid, indicating clearly that a pathway from linolenic to linoleic acid is very minor at most. The distribution of activity in the arachidic acid derived by hydrogenation of the C_{20} fraction was as follows:

$$\underset{1.0}{C_{16}H_{33}}—\underset{3.8}{CH_2}—\underset{60.2}{CH_2}—\underset{7.8}{CH_2}—\underset{27.2}{COOH}$$

First it can be seen that linolenic acid is intermediate between linoleic and γ-linolenic acids in the relative rates of oxidation and incorporation into higher polyenoic acids. Second, some activity is again seen beyond the third carbon. It seems likely that this activity is due to partial degradation and resynthesis of the acids at all stages. The activity in the even carbons cannot be explained at present.

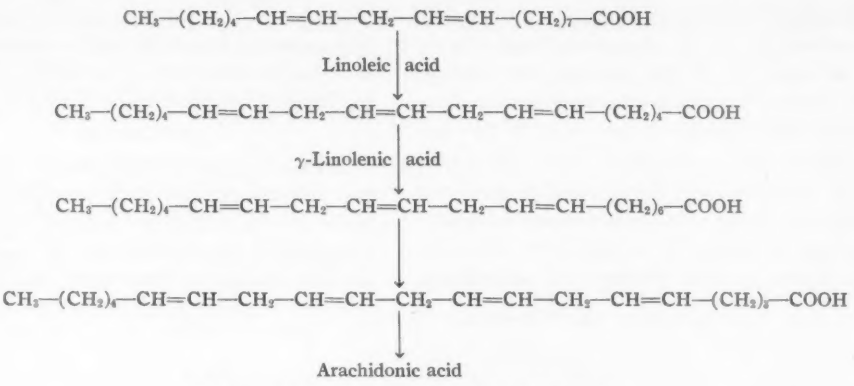

Fig. 2. Pathway of linoleic to arachidonic acid.

Since linolenic acid does not behave as a typical essential fatty acid in feeding experiments, a question arises as to the identity of the active C_{20} acid formed from it. Several observations would indicate that it is not arachidonic acid. First, although chromatography did not completely separate the unsaturated acids, a partial separation was made in which the leading portion (containing docosapentaenoic or docosahexaenoic acids) was arbitrarily separated from the trailing portion (containing the arachidonic acid). When this was done, it was found that the radioactivity was concentrated in the leading portion. Second, alkali isomerization experiments showed that more pentaene and hexaene were formed in the rats which had been fed linolenate. Finally in a separate experiment, fat-deficient rats were fed methyl linolenate and their organ and depot fatty acids were analyzed chromatographically.[18] The fatty acids of rats on the fat-free diet contained no 22-carbon acids whereas those from the linolenate-fed rats contained an appreciable amount of these acids but gave no evidence for linolenic acid. Evidently, the linolenate was rapidly converted to a C_{22} acid. A proposed metabolic pathway for linolenic acid is illustrated in Figure 3.

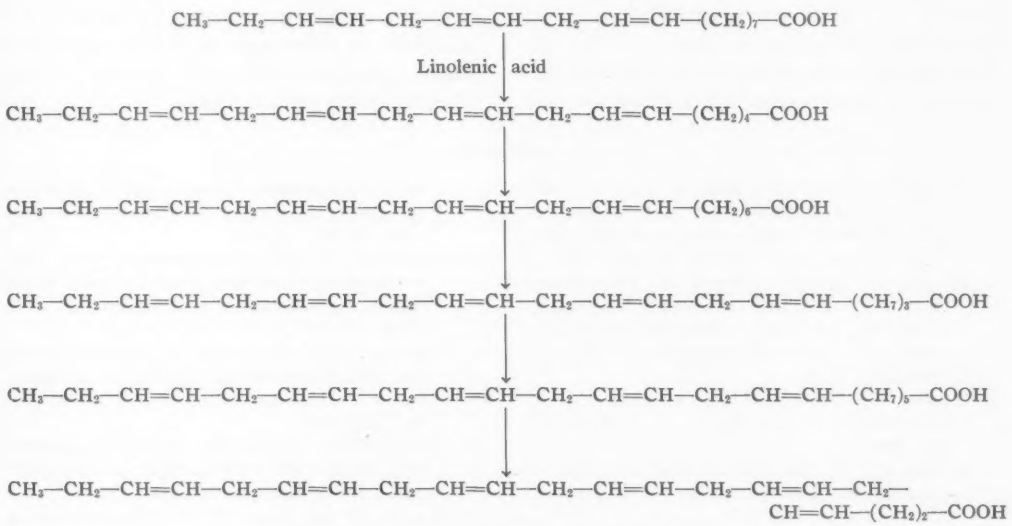

Fig. 3. Proposed metabolic pathway of linoleic acid.

Although these observations serve to explain the earlier data on the changes induced in body fat by ingestion of the dienoic and trienoic acids, they do not explain the appearance of the trienoic acid typical of the onset of the fat-deficiency state. Isolation and characterization of this acid from fat-deficient rats revealed it to be 5,8,11-eicosatrienoic acid.[19] Although it appeared at first that it might be a hydrogenation product of arachidonic acid (the double bond farthest from the carboxy end is lacking), a recent suggestion has been made that it is actually a dehydrogenation product of oleic acid.[20] If this idea proves correct, it seems likely that in the absence of the normal substrates, linoleic or linolenic acids, the unsaturated fatty acid dehydrogenase enzymes use the readily available oleic acid as a substrate. That the product is not an efficient essential fatty acid is attested by the fact that the triene increases concurrently with the severity of the fat-deficiency symptom. In any event, a metabolic pathway may now be proposed similar to those probably followed by linoleic and linolenic acids (Figure 4).

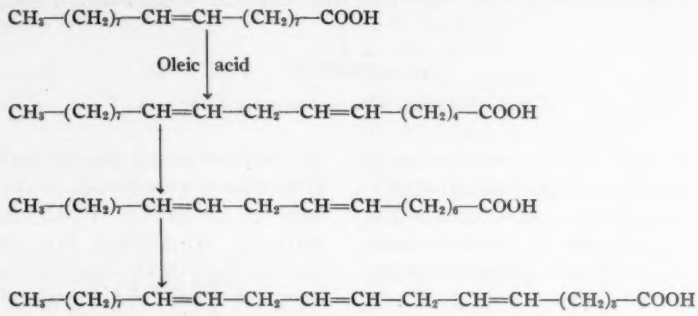

Fig. 4. Proposed pathway of oleic acid to a trienoic acid.

CONCLUSION

If these hypotheses are shown to be correct, the polyunsaturated fatty acids of the animal body can be said to be formed by successive additions of double bonds in the divinyl methane relationship to the existing double bonds of unsaturated fatty acids ingested or synthesized from saturated acids in the body. The new double bonds are introduced between the existing double bonds and the carboxy groups of the acids which may be lengthened by addition of acetate when the double bond system approaches to within three or four methylenes of the carboxy group. There thus arise three "families" of polyunsaturated fatty acids, not readily interconvertible and possibly serving different functions in the body.

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Transport and Metabolism of Chylomicra

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CHYLOMICRA are emulsified fat particles less than one micron in diameter which appear in intestinal lymph during fat absorption. They consist chiefly of triglycerides (80 to 95 per cent). This term has also been applied to plasma lipoproteins of similar composition and structure found in post-absorptive plasma in certain pathologic states (nephrotic syndrome, diabetic acidosis, idiopathic hyperlipemia). For purposes of clarity the latter will be referred to as very low density lipoproteins in the following discussion.

The bulk of the fatty acids of food fats have more than 12 carbon atoms. After absorption such fatty acids appear chiefly in intestinal lymph,[1] whereas only a small fraction of fatty acids with fewer than 12 carbon atoms appear there.[2] Regardless of their chain length, most of the newly absorbed fatty acids in the lymph are incorporated in chylomicron triglycerides.[3] The lipid constituents of lymph other than chylomicron lipid show little change during fat absorption.[4] Absorbed fatty acids which do not enter the intestinal lymphatics apparently are transported to the liver via the portal system. Short chain fatty acids, which are chiefly absorbed by this route, are transported in the unesterified form, as shown by the presence of higher levels of unesterified fatty acid radioactivity in the portal vein than in the vena cava of rats absorbing carbon-14 labeled decanoic acid.[5] Studies utilizing dogs with thoracic duct fistulae to divert chylomicra from the blood stream have shown that small quantities of palmitic acid-1-C^{14} are also absorbed directly into the portal capillaries. In these animals palmitic acid radioactivity was two to five times higher in the portal blood than in blood obtained simultaneously from the vena cava.[6] The data available are compatible with the concept that the distribution of absorbed fatty acids between the intestinal lymph and portal capillaries is largely determined by the extent to which newly absorbed fatty acids are incorporated into triglycerides in the intestinal mucosa.[2]

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CHEMICAL COMPOSITION

The chemical composition of chylomicra obtained from blood plasma of humans after ingestion of fats of widely varying chemical composition, such as eggs, cream, and safflower oil, is relatively constant.[7] In several animal species studied, the phospholipid content of the chylomicra ranged from 3 to 10 per cent, while the content of cholesterol and its esters varied from less than 1 to 8 per cent (see Table I for representative examples). Protein content was usually 1 to 2 per cent, although as little as 0.3 per cent protein has been found in chylomicra obtained from rats absorbing large quantities of olive oil.[8] Since such chylomicra remain finely emulsified, their content of phospholipids, rather than their content of protein, is probably chiefly responsible for maintenance of their integrity. Indeed, it has been shown that chylo-

TABLE I

Per Cent Composition of Lymph Chylomicra

Species	Fat fed	Cholesterol esters	Unesterified cholesterol	Phospholipids	Tri-, di- and monoglycerides	Protein
Dog	Cream	0.9	0.7	6.1	91.5	0.8
Rat	Olive oil	0.1	0.2	4.4	95.0	0.3
Man	Mixed	4.1	0.8	7.5	85.9	1.7

micra flocculate upon incubation with lecithinase.[9] The recent report that chylomicra may contain small quantities of di- and monoglycerides[10] suggests that these compounds may also contribute to the stability of chylomicra. The more polar lipid constituents of chylomicra (unesterified cholesterol and phospholipids) have been shown to exchange rapidly with like constituents of higher density plasma lipoproteins *in vitro*.[11,12] This indicates that these moieties are present at or near the interface between chylomicra and the surrounding aqueous medium, in accordance with established principles of the structure of emulsions.

QUANTITATION OF CHYLOMICRONEMIA

Chylomicra are responsible for the lactescence of blood plasma after ingestion of fat. In healthy humans lactescence appears one to two hours after the intake of appreciable quantities of food fat (0.5 to 1.5 g/kg body weight), reaches a peak in three to four hours, and disappears after five to six hours. Determination of plasma triglyceride concentrations reveals the same pattern. It has been claimed that chylomicron counts provide an index of the concentration of newly absorbed fatty acids in the rat.[13] Such counts, however, measure only the larger chylomicra. Smaller particles, "lipomicra," cannot be estimated by this method. With the exception of the use of radioactive tracers, such as carbon-14 labeled fatty acids, the most reliable estimates of chylomicron concentration are based on centrifugal separation of chylomicra followed by chemical analysis of the separated material. Turbidimetric measurement of lactescence is not a reliable indicator of chylomicron concentration as determined by chemical analysis of plasma or very low density (less than 1.006) lipoprotein triglycerides, presumably because mean particle size varies in different individuals and at different times after the ingestion of fat (Fig. 1).* Likewise, plasma nephelometric measurements correlate only partially with chylomicron counts.[14]

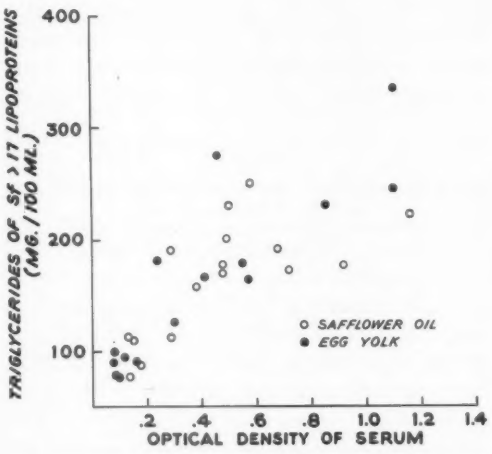

Fig. 1. Relation between optical density and total lipids of very low density lipoproteins of blood serum from healthy young adults at various intervals following ingestion of fat-rich meals.

ROLE OF LIPOPROTEIN LIPASE

The mechanism by which chylomicra are removed from the blood has been the subject of considerable speculation since the discovery that the visible lipemia produced in dogs by fat feeding could be made to disappear rapidly by the injection of small quantities of heparin.[15] It is now established that heparin acts by causing release into the circulation of a lipoprotein lipase which catalyzes the hydrolysis of the chylomicron triglycerides to glycerol and fatty acids.[16] This property of heparin is shared by other natural and synthetic heparinoids, including inorganic materials, which have the common property of high molecular weight and high density of negative charge.[17,18] The production of fatty acids *in vitro* is paralleled by a decrease in the turbidity of the post-heparin plasma.[16] Monoglyceride concentration rises

* There is no question that lactescence of plasma is usually associated with increased concentrations of triglycerides relative to other lipids. However, an association of lactescence with relatively low concentrations of phospholipids and protein is even more striking. Sera obtained from cholesterol-fed rabbits may be markedly lactescent in spite of negligible proportions of triglycerides in the constituent lipoproteins. Cholesterol esters are the predominant lipid constituents and phospholipid and protein concentrations are relatively low.[25] It is likely that the relative abundance of phospholipids and proteins determines the maximal surface to volume ratio possible for lipoprotein molecules, and, thus, molecular size.

during the clearing process, whereas little accumulation of diglycerides is found.[10] The fatty acids released by hydrolysis combine with albumin to form a complex which, in effect, removes the fatty acids from the reaction medium.[19,20] *In vivo*, the fatty acids are rapidly removed from the plasma[11,21] so that the net result is not only clearing of the lactescence, but also accelerated removal of chylomicron fatty acids from the circulation. Lipoprotein lipase has been extracted in high yield from adipose tissue, in moderate yield from heart muscle, and in small quantities from lung and skeletal muscle. Little or no activity has been found in extracts of other tissues, including liver.[16,22]

Several lines of indirect evidence indicate that the clearing reaction plays a central role in the disposition of chylomicra, although its physiologic significance in this process is not clearly established. Since protamine sulfate, an anti-heparin agent, inhibits the *in vitro* lipolytic activity of post-heparin plasma[23] and of lipoprotein lipase in tissue extracts,[16] the clearing enzyme may depend upon heparin or heparin-like substance for its activity. Bacterial "heparinase," moreover, inactivates lipoprotein lipase extracted from chicken adipose tissue.[24] The observation that protamine and other anti-heparin agents decrease the rate of removal of chylomicra from the blood *in vivo*[25] suggests that triglyceride hydrolysis catalyzed by lipoprotein lipase is an integral part of the removal mechanism. Clearing activity is found in one strain of rats during fat absorption, and the extent of the activity *in vitro* is sufficient to account for the hydrolysis and removal of an appreciable portion of triglycerides delivered to the plasma during fat absorption.[26] However, such high levels of enzyme activity have not been observed in other strains of rats during alimentary lipemia.[21]

In man, small quantities of lipoprotein lipase are found in fasting plasma.[27–30] A slight but significant increase in lipolytic activity may be noted after ingestion of large quantities of cream (Table II). The magnitude of the activity found after the ingestion of fat is sufficient to account for the intravascular hydrolysis of only about 1 per cent of the ingested fat in a period of eight hours.[30]

The studies of Carlson and Wadström in man showed that mono- and diglycerides constitute about 10 per cent of the neutral glycerides of plasma in the fasting state and during alimentary lipemia.[10] Mead and Fillerup demonstrated that mono- and diglycerides constitute about 20 per cent of newly absorbed fatty acids in the rat.[31] It is not necessary to assume that the partial glycerides were produced by intravascular hydrolysis of triglycerides, since it is possible that they were original constituents of the chylomicron[10] or were returned to the circulation after extravascular hydrolysis.

Further information regarding the role of triglyceride hydrolysis in chylomicron disposition has been gained from studies using chylomicra obtained from thoracic duct lymph of dogs fed palmitic acid labeled with carbon-14.[21] The chylomicra, whose radioactivity was contained almost exclusively in the triglyceride moiety, were transfused into fasting dogs. They disappeared exponentially with time from the plasma, with a half-time of about 15 minutes. Radioactivity appeared rapidly in the unesterified fatty acids of the plasma, and their specific radioactivity rapidly reached a level exceeding 50 per cent of the radioactivity of the fatty acids in the chylomicron triglycerides. The unesterified fatty acids disappeared from the plasma very rapidly, with a half-time of about two minutes. From these data it was possible to calculate that a considerable portion of the radioactivity which disappeared from the chylomicra appeared in the unes-

TABLE II

Effect of Ingestion of Cream or Glucose on Lipolytic Activity of Human Plasma

	Unesterified fatty acid release* $\mu eq/ml/hr$		
	Fasting	3½ hrs after meals	Change and standard error
Cream	0.044	0.108	$+0.064 \pm 0.020$ ($p < 0.01$)
Glucose	0.024	0.022	-0.002 ± 0.020 ($p > 0.9$)

* Mean values for 12 medical students; plasma incubated with 0.1 volume of 1 per cent cocoanut oil emulsion at 38° C.

terified fatty acids during the removal process.*

A final piece of evidence supporting the concept that lipoprotein lipase is concerned with the removal of chylomicra from the plasma was supplied by a study of three siblings with a clinical picture of idiopathic hyperlipemia.[32] On a normal diet, these individuals manifested a pronounced chylomicronemia after an overnight fast. When fat was omitted from their diet, the chylomicronemia disappeared in about three days and the concentration of plasma triglycerides fell sharply. Ingestion of a single fat-rich meal produced a chylomicronemia which persisted for two or three days. Very little lipoprotein lipase activity was found in the plasma of these subjects after injection of large amounts of heparin, and the long-term administration of heparin to one of the siblings failed to reduce the chylomicronemia produced by the intake of 1 g of fat/kg body weight daily. It is likely that the persistent chylomicronemia observed in these individuals was related to deficiency of lipoprotein lipase in their tissues.

FATE OF CHYLOMICRA

The sites of removal of chylomicra from the blood have also been investigated in recent years. The obvious anatomic fact that chylomicra bypass the liver by first entering the general circulation suggests that they may be removed by peripheral tissues. French and Morris noted that the rate of removal of intravenously administered chylomicra in rats was inversely proportional to the quantity administered and that small chylomicron particles were removed more slowly than larger particles.[33] These kinetic observations are similar to those pertaining to removal of particulate material by the reticuloendothelial system.[34] Measurements of tissue radioactivity at intervals after administration of rat chylomicra labeled with carbon-14 palmitate showed that tissue radioactivity was predominantly in the liver and adipose tissue.[35] Byers and Friedman reported that newly absorbed cholesterol, which is known to be transported chiefly in the form of chylomicra,[11,36] appears to be localized in the Kupffer cells of the liver.[37] The significance of this observation is open to question, however, in view of the rapid exchange of the unesterified cholesterol of chylomicra with the cholesterol of higher density lipoproteins.[11]

The studies of Morris and Courtice indicate that chylomicra can escape intact from the circulation of the liver.[38] In cats in which partial ligation of the inferior vena cava above the liver had caused distention of the portal sinusoids, as much as 15 per cent of transfused chylomicra could be recovered intact in liver lymph. Of interest in this connection is Fawcett's demonstration by electron microscopy of discontinuities up to 1 micron in diameter in the sinusoidal epithelium of the liver.[39] Such large pores would allow direct passage of chylomicra into the space of Disse between the sinusoidal epithelium and the hepatic parenchyma. This space is thought to be the site of origin of the lymphatic system of the liver. Studies of the uptake of chylomicra labeled with carbon-14 palmitate in unanesthetized dogs also have indicated that the liver is a major site of chylomicron removal. Uptake by the liver was shown by the significantly lower level of radioactivity in the hepatic vein than in the portal vein or peripheral artery; uptake by tissues of the hind leg and heart was demonstrated similarly.[6]

According to Bragdon and Gordon[40] the sites of chylomicron removal may depend on the nutritional state. They found little radioactivity in adipose tissue of fasting rats killed 10 minutes after the injection of chylomicra labeled with carbon-14 palmitate, but noted considerable adipose tissue radioactivity in similarly treated rats pre-fed large quantities of glucose. The liver showed a high level of radioactivity in both groups of animals. The radioactivity found in adipose tissue could not have been derived from retransported unesterified fatty acids. This was demonstrated by the virtual absence of radioactivity in the adipose tissue of rats, whether pre-fed glucose or not, 10 minutes after injection of unesterified fatty acids. Apparently then, chylomicra can be removed directly from the circulation both in liver and in adipose tis-

* Studies with oleic acid-1-C^{14} have given similar results.[6]

sue without prior intravascular hydrolysis. Whether lipoprotein lipase is actively involved in the removal process in both areas is not known. The presence of the enzyme in adipose tissue and its apparent absence in liver have already been mentioned. Albrink and Man reported that when glucose is fed along with a fat meal to humans, the magnitude of the rise in triglyceride fatty acids and the appearance of visible lipemia are diminished.[41] They suggested that the availability of glucose facilitated rapid removal of absorbed fat by the depots. This finding, although compatible with the findings of Bragdon and Gordon,[40] does not agree with the observation that prefeeding glucose to fasted rats does not alter the rate of removal of intravenously administered chylomicra.[42] It is possible that species differences account for this apparent discrepancy.

The role of other lipoproteins in the removal of chylomicra from the blood is poorly defined. The data of Bergström, Borgström, and Rottenberg[43] indicate that in rats newly absorbed triglycerides do not mix appreciably with endogenous plasma triglycerides, which are present predominantly in the very low density lipoproteins.[44] On the other hand, Jones and co-workers observed transient elevation of lipoproteins having S_f rates above 60 after feeding fat in humans and the subsequent appearance of higher density molecules with flotation rates down to S_f 30.[45] They suggested that such very low density lipoproteins constitute a transport system for absorbed fatty acids. The possibility that alterations in the particle size and composition of chylomicra absorbed at varying times after fat ingestion accounts for this finding has not been ruled out. In dogs both the triglyceride and phospholipid moieties of intravenously administered chylomicra are removed from the very low density lipoprotein-chylomicron fraction (density less than 1.006) of plasma lipoproteins at the same rate.[21] The concentration of phospholipids in high density lipoproteins increases significantly as clearing proceeds.[6] A similar increase in high density lipoprotein phospholipids, involving chiefly the density 1.063–1.120 lipoprotein fraction, has been noted following fat ingestion in man.[6,46] Korn has demonstrated that high density lipoproteins are highly potent activators of artificial fat emulsions used as substrate for tissue lipoprotein lipase.[47] It can be shown that the lipoprotein complexes with the emulsion and that this altered emulsion will then complex readily with lipoprotein lipase, whereas untreated fat emulsions complex relatively poorly with the enzyme.[48] These observations all suggest that high density lipoproteins may constitute an integral part of chylomicron structure, and thus make chylomicron triglycerides available for hydrolysis by lipoprotein lipase. Observations concerning the nature of the protein moiety of chylomicra further substantiate the relation between chylomicra and high density lipoproteins. Rodbell has recently shown that both human chylomicra and high density lipoproteins have aspartic acid, serine, and theronine as N-terminal amino acid end groups.[49] Since glutamic acid is also N-terminal in S_f 0–100 lipoproteins, it is apparent that these lipoprotein classes do not form part of a "spectrum" which includes chylomicra. In addition, Rodbell and Fredrickson have shown that, in the dog, chylomicra contain two distinct proteins with N-terminal glutamic and aspartic acid, respectively. The latter is identical with the protein of high density lipoproteins.[50]

The mechanism by which chylomicra are removed from the circulation is a matter of conjecture. In the liver the chylomicron may be phagocytosed by a Kupffer cell, or it may pass through a sinusoidal pore and present itself directly to the hepatic parenchymal cell. In adipose tissue an apparently intact capillary wall is interposed between the chylomicron and the adipose tissue cell. At this site hydrolysis of chylomicron triglyceride may occur at the surface of the capillary endothelium; the liberated fatty acids would then be transported across the capillary and presented to the adipose tissue cell to be accepted or rejected, depending upon the availability of carbohydrate for cellular metabolism. Alternatively, a specific mechanism may be responsible for the transfer of chylomicron triglyceride across the capillary wall, and hy-

drolysis may take place at the surface of the tissue cell. Shapiro and co-workers have shown that labeled stearic acid can be taken up by rat adipose tissue *in vitro* when presented as the albumin complex.[51] Uptake was promoted by refeeding starved animals, and inhibited by cyanide. Tissue radioactivity was present chiefly in triglycerides. Adipose tissue can also remove triglycerides from plasma during *in vitro* incubation.[52] This uptake, unlike that of fatty acids, is inhibited by fluoride, as well as by cyanide. The significance of these observations in terms of chylomicron triglyceride transport is not yet clear.

The subsequent fate of chylomicron triglyceride fatty acids is similar to that of unesterified fatty acids injected intravenously as an albumin complex.[11] These fatty acids apparently enter a fatty acid pool which is predominantly intracellular and which is approximately 100 times greater in magnitude than that of the plasma. A major fate of this pool is rapid oxidation to carbon dioxide and water. The oxidation of chylomicron triglyceride fatty acids is spared by the ready availability of carbohydrate.[53] In fasted rats, however, chylomicron triglyceride fatty acids are as readily available as glucose for oxidation by peripheral tissues, and glucose does not appear to be oxidized preferentially in this situation.[53]

The subsequent fate of other chylomicron constituents is not known. The rise in high density lipoprotein phospholipids during removal of chylomicra from the circulation[6] suggests that at least a portion of the chylomicron residue left after hydrolysis of the triglycerides is returned to the circulation as high density lipoprotein. The rapid exchange of chylomicron cholesterol and phospholipids with other lipoproteins[11,12] precludes their use as tracers in this problem.

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The Metabolism of Plasma Unesterified Fatty Acid

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THE existence of small quantities of unesterified fatty acid in blood plasma has been known for several years. In particular, the finding of unesterified fatty acid (UFA) in albumin fractions prepared from fresh human plasma by the method of Cohn, Hughes, and Weare,[1] and the report of Davis[2] that fresh serum yielded small quantities of UFA, indicated that UFA actually exists as such in plasma and is not a laboratory artifact. The possible metabolic significance of plasma UFA remained unappreciated, however, until studies of the lipemia-clearing reaction indicated that this reaction involves a process of lipolysis, and that a principal product of lipemia clearing *in vitro* is unesterified fatty acid.[3–6] In 1956, similar and independent reports from two laboratories[7,8] demonstrated that plasma UFA levels are consistently responsive to nutritional status and to hormonal administration, and the hypothesis was advanced that UFA might represent a lipid fraction primarily concerned with the supply of fat to tissues for oxidative metabolism. Since then, further studies have considerably extended our knowledge of the metabolism of plasma UFA. It will be the purpose of this paper to summarize and illustrate these studies, and to present our present concepts of the metabolic role of plasma unesterified fatty acids.

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It should be stated at the outset that the study of UFA metabolism has been greatly facilitated by the development of more precise analytic technics for the measurement of plasma UFA levels. Limitations of space do not permit a detailed description of methodology here, but methods are available for measuring plasma UFA concentration with a standard error of 0.02 meq/liter. Of the methods currently available, the one employed by Dole[8] has the advantage of greater convenience and rapidity, but is somewhat less accurate than the method employed by Gordon,[9] since lactate interferes appreciably with the results obtained by the former method.

METABOLIC OBSERVATIONS

Although the plasma UFA concentration is subject to many influences, the normal post-absorptive concentration is of the order of 0.5 meq/liter. This concentration is rapidly depressed by the ingestion of carbohydrate. Figure 1 illustrates the response of plasma UFA level to glucose administered orally. In every case the UFA level decreased by 30 min, usually reaching a minimum at 2 hr. The results of many such experiments are summarized in Figure 2. The effect of glucose illustrated here is the average of the separate experiments shown in Figure 1, and each of the curves in Figure 2 represents the average of several experiments. The feeding of any of the three amino acids, alanine, leucine, or glutamic acid, resulted in a qualitatively similar but quantitatively smaller effect on plasma UFA concentration. In contrast, fasting characteristically results in a slow steady rise in plasma UFA concentration.

The UFA level is also highly sensitive to the administration of a number of hormones.

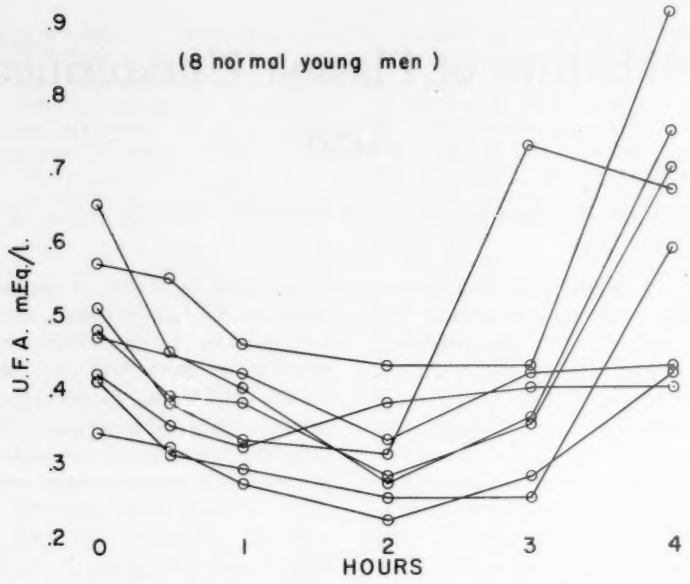

Fig. 1. Response of UFA to oral glucose. (From GORDON, R. S., JR. and CHERKES, A.: *J. Clin. Investigation* 35:206, 1956.)

This is illustrated in Table I, which describes the effects of the intravenous administration of 7 units of regular insulin, 1 mg of glucagon, or 0.1 mg of epinephrine on plasma UFA concentration. Insulin results in a rapid decrease in UFA level. Epinephrine results in an equally dramatic rise. Glucagon results in a more sustained decline in UFA level.

TABLE I

The Effect of Hormonal Administration on Plasma UFA Concentration*

Time *a.m.*	Epinephrine *0.1 mg*	Glucagon *1 unit*	Insulin *7 units*
9:00	0.145	0.264	0.381
9:15	0.252	—	0.267
9:30	0.302	—	0.207
9:45	—	0.160	0.346
10:00	0.197	—	0.352
10:20	—	0.119	—
10:55	—	0.182	—

* Each agent given at 9:00 a.m. after an overnight fast; UFA concentration expressed in meq/liter.

The question of the metabolic function of plasma UFA therefore arises. The available evidence, some of which will be presented here, permits a fairly conclusive answer: that plasma UFA is a transport form of lipid which is readily available to most tissues as a substrate for oxidation, and that it is the major fuel from which energy is derived in the post-absorptive state.

The evidence for this concept comes from two main lines of experimentation. The first of these lines of evidence is derived from measurements of arteriovenous UFA differences across several organs. In the course of cardiac catheterization it is often possible to obtain simultaneous samples from an artery and from the coronary sinus, or from an artery and from the hepatic vein. Measurements of the UFA levels of such samples yield the A-V UFA difference across the myocardium and the liver respectively. Figure 3 summarizes the results, for fasting subjects, of many such experiments. Included in this figure are the averages of the results obtained with 12 samples from the coronary sinus, 11 from the hepatic vein, 8 from the greater saphenous vein, 2 from the internal jugular vein, 4 from the pulmonary artery, and 3 from the venae cavae (each, of course, accompanied by a

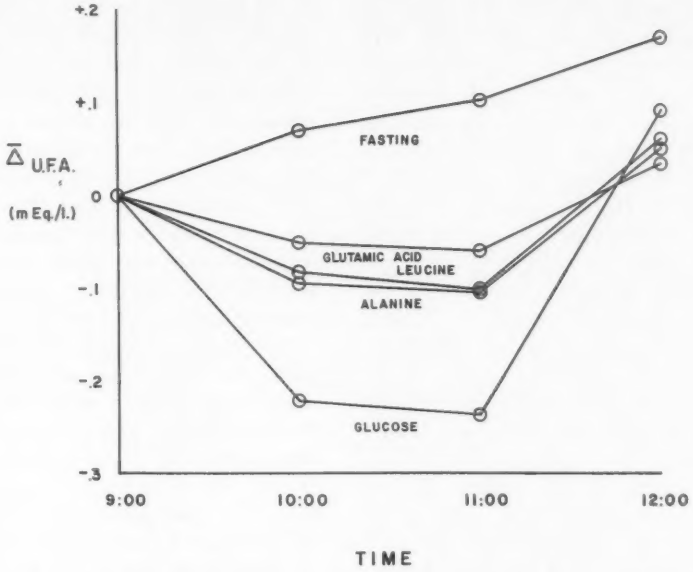

Fig. 2. Effect of feeding on unesterified fatty acid. (From GORDON, R. S., JR.: *J. Clin. Investigation* 36:810, 1957.)

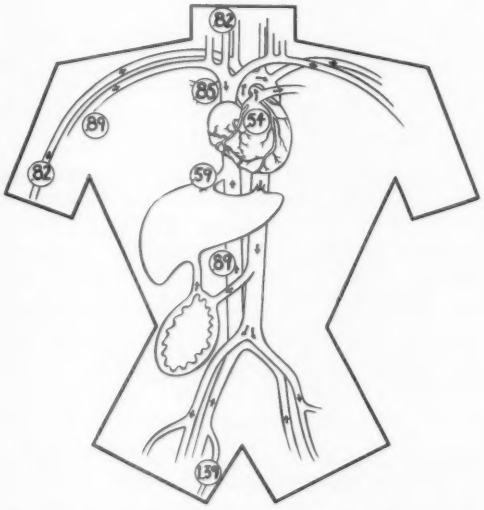

Fig. 3. Arterial and venous UFA levels for A-V differences. (From ANFINSEN, C. B., JR.: *Fed. Proc.* 15:894, 1956.)

simultaneous arterial sample). None of the subjects had demonstrable metabolic disease, and the patients undergoing cardiac catheterization were free from clinically apparent congestive failure. The average arterial UFA level for all these subjects was 0.82 meq/liter. In those subjects in whom coronary sinus blood was obtained, there was an average A-V difference of 0.28 meq/liter. This finding is represented in the figure by indicating 0.54 as the average coronary sinus UFA level. The magnitude of the A-V difference is large (0.28 meq/liter) compared with experimental error; the myocardium is hence obviously extracting UFA from perfusing blood. A similar conclusion can be drawn for the liver, for the average hepatic A-V UFA difference was 0.23. In contrast, the two measurements of arteriovenous UFA differences in the cerebral circulation (internal jugular vein samples) revealed no A-V difference. This is consonant with the belief that brain tissue does not oxidize appreciably quantities of fat.

The source of the UFA's which are being extracted by such tissues as the heart and liver has been revealed by measurements of the UFA concentrations of samples obtained by catheterization of the greater saphenous vein. This vein drains primarily the subcutaneous adipose tissue of the thigh. It also

drains the skin, but skin blood flow is relatively small, and valves prevent admixture with blood from the deeper tissues. Saphenous vein samples from fasting subjects all revealed large negative A-V differences. The average saphenous vein value for 8 samples was 1.39 (Fig. 3), with an average A-V difference of −0.57 meq/liter. It thus appears that the adipose tissue is supplying UFA to the blood, and the other tissues are extracting the UFA. It should be noted that the extraction by the viscera is probably greater than that indicated by the measured hepatic vein A-V difference, because omental fat may be expected to be adding UFA to the blood within the enteric circulation at the same time that other tissues are extracting it.

Now this is true in the fasting subject only. In the carbohydrate-fed subject the UFA level not only decreases but the A-V difference is abolished. This has been demonstrated by administering glucose (100 g) and insulin (0.1 unit/kg body weight) to subjects while obtaining sequential samples for the measurement of A-V UFA differences. Table II illustrates the results of six such experiments. In every case there occurred, coincident with a decreased level of UFA, complete cessation of net transport of UFA as indicated by the abolition of the A-V differences. The observed events indicate that the transport of fat as UFA is controlled primarily by the adipose tissue, and that when glucose is made available there is first a decrease in the liberation of UFA from depot fat, a resultant drop in arterial UFA concentration, and then a cessation of extraction by other tissues. The reverse sequence is not reasonable, as in that case the arterial level would be expected to rise as tissue extraction was halted.

The concept emerges, therefore, that adipose tissue, far from being an inert depot, represents tissue with a high metabolic activity which is subject to careful homeostatic control. The mechanism of this control is as yet unknown. In order further to test this concept, however, experiments have been conducted on the effects of nutritional status and hormonal administration upon isolated adipose tissue fragments incubated *in vitro*.[10] The adipose tissue used in these studies was taken from the epididymal fat bodies of young male rats weighing about 200 g. The tissue was incubated with gentle rocking in a medium of Krebs-Ringer phosphate buffer containing 5 per

TABLE II

Abolition of A-V Differences in UFA Concentration by Intravenous Glucose and Insulin*

Patient	Venous site	Glucose and insulin	UFA (meq/liter)		
			Arterial	Venous	Difference
J.B.	H.V.	Before	0.68	0.63	0.05
		After	0.10	0.10	0.00
W.H.	H.V.	Before	0.89	0.78	0.11
		After	0.25	0.25	0.00
A.J.	H.V.	Before	0.42	0.31	0.11
		After	0.08	0.08	0.00
G.E.	S.V.	Before	0.46	1.09	−0.63
		After	0.16	0.19	−0.03
R.S.	S.V.	Before	0.39	0.82	−0.43
		After	0.22	0.20	0.02
I.S.	S.V.	Before	0.36	0.50	−0.14
		After	0.13	0.12	0.01

* Samples from hepatic (H.V.) and saphenous (S.V.) veins. The figures are means of duplicate analyses.

(From GORDON, R. S., Jr.: *J. Clin. Investigation* 36: 810, 1957.)

TABLE III

UFA Production from Tissues of Fasted and Fed Rats

State	UFA production (μeq/g tissue/hr)	
	Mean	S.D.
Fasted (4 tissues)	6.4	0.75
Fed (4 tissues)	−0.2	0.38

(From GORDON, R. S., JR. and CHERKES, A.: *Proc. Soc. Exper. Biol. & Med.* 97: 150, 1958.)

TABLE IV

Effect of Epinephrine (0.01 μg/ml) on UFA Production from Tissues of Fasted Rats

Treatment	Mean UFA production (μeq/g tissue/hr)
None (4 tissues)	1.31
Epinephrine (4 tissues)	2.31

Differences between outputs of treated tissues and contralateral controls: Mean 1.00; S.D. 0.62.

(From GORDON, R. S., JR. and CHERKES, A.: *Proc. Soc. Exper. Biol. & Med.* 97: 150, 1958.)

cent bovine serum albumin. After four hours' incubation the medium was analyzed for concentration of UFA. The results of these experiments are shown in Tables III, IV, and V. These experiments show that the rate of

TABLE V

Effect of Insulin (0.01 unit/ml) of UFA Production from Tissues of Fasted Rats

Treatment	Mean UFA production (μeq/g tissue/hr)
None (4 tissues)	1.58
Insulin (4 tissues)	−1.03

Differences between treated tissues and contralateral controls: Mean 2.61; S.D. 0.43.

(From GORDON, R. S., JR. and CHERKES, A.: *Proc. Soc. Exper. Biol. & Med.* 97: 150, 1958.)

evolution of UFA from adipose tissue was significantly increased by fasting the donor animal or by the addition of epinephrine to the medium; it was decreased by the addition of glucose and insulin to the medium or by feeding the donor animal with carbohydrate. It is hence apparent that isolated rat adipose tissue behaves *in vitro* in a manner consistent with the above concept derived from *in vivo* studies with intact human subjects.

In order to determine whether the quantity of UFA extracted by the tissues is metabolically significant, the A-V oxygen difference was measured across the tissue simultaneously with the measurement of the A-V UFA difference. These figures permit the calculation of the percentage of the tissue oxygen uptake which would be required to effect the complete oxidation of the fatty acids simultaneously extracted, assuming an average fatty acid molecular weight of 275, and, further, that the red blood cells contribute no UFA to tissue. This result is known as the oxygen extraction ratio. The oxygen extraction ratios for five coronary sinus samples were found to be 87, 70, 70, 38, and 36 per cent. These values are large, and indicate that UFA extraction is such that it could account for a large fraction of the oxidative needs of the myocardium in the basal state. The oxygen extraction ratios for three hepatic vein samples were 31, 103, and 165 per cent. The liver is the only organ studied that sometimes shows an oxygen extraction ratio of greater than 100 per cent. This is probably due to the fact that the liver makes ketone bodies and hence complete oxidation of fatty acids probably does not occur.

The other main line of evidence which indicates that plasma UFA is an important and immediate substrate for oxidation derives from the results of isotopic UFA turnover studies. Such studies have been conducted in the rat,[11] dog,[12,13] and in man, [14,15] but the subsequent discussion will be limited to those results obtained with human subjects. In these studies a tracer amount of labeled fatty acid bound to albumin was injected intravenously into normal human subjects. The fatty acid was labeled with C^{14} in the carboxyl group. Serial arterial blood samples were collected at frequent intervals, and the UFA specific activity determined. After the first hour, the indwelling arterial needle was removed, and subsequent samples collected at less frequent intervals from a peripheral vein. Simultaneously, the expired CO_2 was collected in serial samples and its specific activity determined. The results of a typical experiment, using palmitate-1-C^{14}, are shown in Figure 4, in which there is plotted the logarithm of the specific activity, for UFA and for CO_2, against time.

It was found that the UFA specific activity decreased very rapidly at first, and then declined at a much slower rate for several hours. A straight line can be fitted to that portion of the curve along the points obtained during the first 4 to 10 min. If we assume that this represents a time when the departure of UFA-C^{14} from the plasma is unaffected by the return of UFA-C^{14} to the plasma from the secondary pool or pools with which it is initially equilibrating, then an initial disappearance rate can be estimated from the slope of this line. The half time for UFA during this initial period is of the order of 2.5 to 3 min. Furthermore, by extrapolating this portion of the curve back to zero time we should obtain an estimate of the size of the initial pool in which the injected UFA is distributed, namely the plasma volume. That

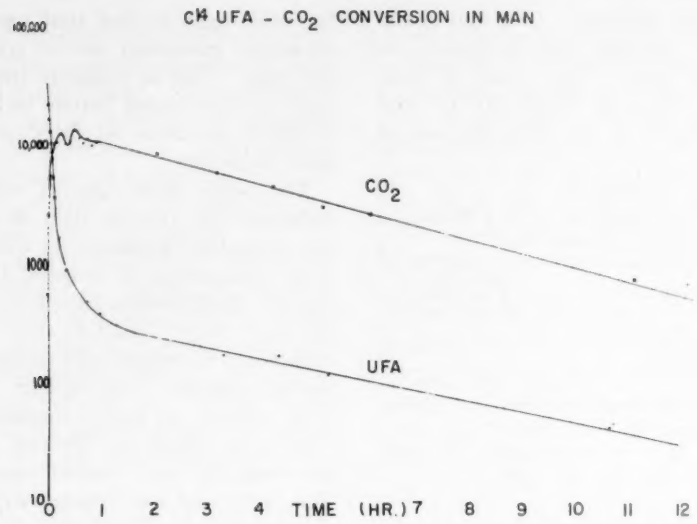

Fig. 4. The disappearance of palmitate-1-C^{14} from plasma and the appearance of $C^{14}O_2$.

this is indeed the case is illustrated by the data in Table VI.

TABLE VI

The Initial Disappearance of Palmitate-1-C^{14} from Human Blood Plasma of Fasting Subjects

	V.W.	M.B.
UFA (meq/liter)	0.89	0.61
Initial T-1/2 (min)	3.4	2.6
Pool size (meq)	3.1	1.7
Volume of distribution (liters)	3.5	2.7
UFA flux from plasma (meq/24 hrs)	890	650

The appearance of labeled CO_2 derived from palmitate-1-C^{14} is relatively rapid, with peak specific activity obtained in about 25 min. Following this the logarithmic curve of CO_2 specific activity vs. time declines at a rate which is very similar to that of the UFA specific activity curve. The rapid appearance of the isotopic carbon in the expired CO_2 gives evidence that the disappearance of UFA from the plasma makes the material readily available to the enzyme systems responsible for the oxidative degradation of fatty acids. Undoubtedly this process is an intracellular one; there is excellent evidence that the enzymes necessary for fatty acid oxidation reside in mitochondria. It follows, therefore, that cellular membranes are readily permeable to UFA, in spite of its strong binding to serum albumin (*infra vide*). The shape of the curve for the disappearance of labeled UFA from plasma, with a slower component of disappearance becoming evident after the first rapid one, makes it evident that some reflux of this intracellular fatty acid into the plasma may occur.

The simplified picture shown in Figure 5 may hence be constructed from these data. Labeled UFA injected into plasma rapidly enters a secondary intracellular "pool" or "pools," from which some re-entry into plasma can occur. Oxidation within this "pool" gives rise to labeled CO_2.

The question that we would most like to answer on the basis of these turnover experiments, namely the quantitative estimation of the contribution of the oxidation of UFA to whole body energy metabolism, should be answerable on the basis of these data. Of course, experimental errors prevent the determination with great precision, but it should be possible to discover whether or not UFA oxidation contributes as much to the energy

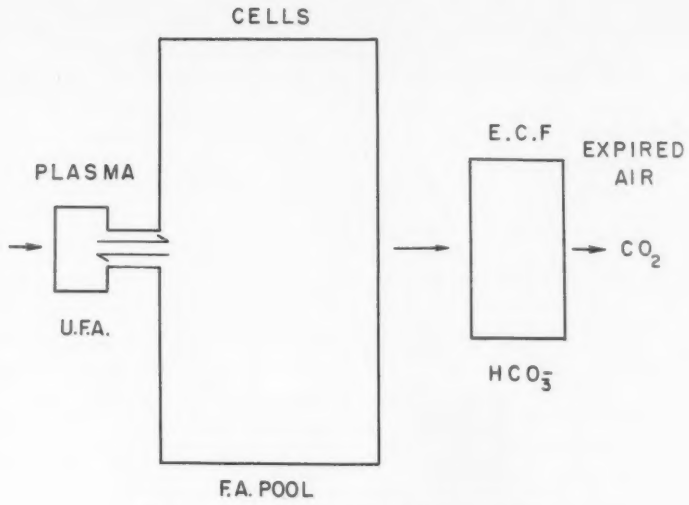

Fig. 5. A simplified scheme of UFA metabolism.

metabolism of the organism as a whole as it appears to contribute to the metabolism of those tissues which can be studied by means of A-V differences. The mathematical treatment of these turnover data has not yet been completed, but it is possible to say in a preliminary fashion that the process of conversion of UFA to CO_2 in fasting human subjects liberates energy in the range of hundreds of calories per day. The upper limit may be ascertained easily; it is determined by the total outward flux of UFA from the plasma compartment, as noted in Table VI, times the energy equivalence of fatty acids of the usual molecular weights (16 and 18 carbons). This energy equivalence is very close to 2.5 cal/meq. On this basis, the 2 subjects in Table VI were receiving not over 2,200 and 1,600 cal/day, respectively, from UFA oxidation at the time of the procedure. The net rate of removal of UFA from the plasma equals the flux noted in the early minutes of a turnover test minus the rate of re-entry of UFA from tissues back into the plasma compartment. This latter rate is difficult to evaluate with accuracy, and more exact delineation of the role of UFA in over-all energy metabolism will have to await the completion of these calculations.

These considerations have involved the assumption that the oxidation of the labeled carboxyl carbon of palmitic acid-1-C^{14} to CO_2 is indicative of complete oxidation of the entire palmitic acid molecule. This assumption is validated for rats by the study of Weinman *et al.*,[16] which demonstrated that the appearance of $C^{14}O_2$ is identical in rate and amount when the palmitic acid molecule (administered as a tripalmitin suspension) is labeled in either the 1, the 6, or the 11 position. The apparent identity of these three positions indicates that, once initiated, the oxidation of a given palmitic acid molecule proceeds immediately to completion.

Let us now turn to a consideration of the state in which UFA exists in plasma. In the plasma these fatty acids do not exist free in solution, but rather bound to serum albumin. This fact derives from many lines of evidence and can be illustrated by the experiment shown in Figure 6. This figure shows the results of a paper electrophoresis of rat serum, to which a small quantity of labeled palmitate had been added. The curve above the paper strip is a radioautograph of the paper strip. The radioactivity is found almost exclusively associated with the albumin fraction. The unesterified fatty acids are bound to albumin and travel with it on electrophoresis.

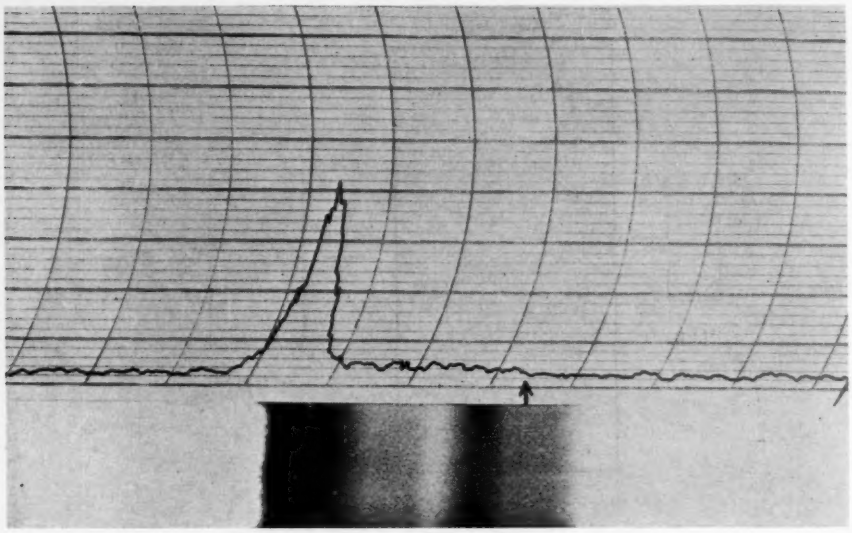

Fig. 6. Paper electrophoresis of rat serum with sodium palmitate-1-C^{14}. (Barbital buffer pH 8.6.)

The actual substance which displays the metabolic properties herein described is therefore a complex between UFA and serum albumin. Recent studies of the interaction of human serum albumin with long-chain fatty acid anions have defined the nature of this complex at physiologic pH and ionic strength.[17] These studies were made possible by the development of an albumin preparation essentially free of fatty acids.[18] The method employed consisted in the equilibration of increasing amounts of each fatty acid being studied between two phases, n-heptane and an aqueous solution of serum albumin in phosphate buffer, pH 7.4 and ionic strength 0.16. The concentration of fatty acid *not* bound to albumin in each aqueous phase was determined from the concentration in heptane, using the results of identical distribution experiments performed in the absence of albumin. The principle behind this method is that two fatty acid solutions, one with and one without albumin, in equilibrium with the same concentration of fatty acid in heptane are in equilibrium with each other. For two such solutions, therefore, the concentration (activity) of the fatty acid in the solution without albumin equals the free (unbound) fatty acid concentration in the solution with albumin.

By performing these experiments over a wide range of fatty acid concentrations it is possible to determine the number of sites on the albumin molecule to which fatty acid ions may bind, and the association constants for the interactions. One may therefore answer the questions of how many UFA's can bind to albumin, and how tightly they are bound. The manner in which this is done may be illustrated as follows. Consider the case of the interaction of a small ion, A, with a protein, P, which has only one site on it to which A can be bound. In this case, the equilibrium reaction is $A + P = AP$, and this is characterized by an equilibrium constant: $k = (AP)/(A)(P)$. Now define the letter $\bar{\nu}$ as the average number of A ions bound per protein molecule. $\bar{\nu}$ therefore equals (AP) divided by the sum of all the forms of P, namely (P) + (AP), i.e., $\bar{\nu} = (AP)/[(P) + (AP)]$. Substituting for (AP) from the expression for k, and dividing by (P) then results in the following equation: $\bar{\nu} = k(A)/[1 + k(A)]$. If we have n identical sites to which A can bind on each protein molecule, the expression for $\bar{\nu}$ is identical with

equation 1, except that the right hand side of the equation is multiplied by n. This can be seen intuitively, for if P is the protein concentration, then the concentration of sites is nP, and the average number of A ions bound per protein molecule will be n times the average number bound per site (which is the above equation).

If we have more than one class of binding sites, with n_1 identical sites in the first class, n_2 identical sites in the second class, and so on, the equation for $\bar{\nu}$ is of the same general form, except that there is a separate term for each class of sites. The general equation for the binding of A to several classes of sites on a protein molecule may hence be formulated as:[19]

$$\bar{\nu}_A = \sum_i \bar{\nu}_{Ai} = \sum_i \frac{n_i R'_{Ai} c_A}{1 + k_{Ai}' c_A} \qquad \text{Equation 1.}$$

in which k'_{Ai} is the association constant for each of the n_i sites in class i under any particular set of conditions, and c_A is the free (unbound) concentration of A in equilibrium with the protein.

Inspection of equation 1 reveals the important fact that the two parameters which are related to each other are $\bar{\nu}$ and c_A. The total protein concentration and the total concentration of A do not enter into the equation; only the average number of A molecules bound per protein molecule (or the average number of moles of A bound per mole of P) is important.

Experimentally, therefore, in order to study the binding of a small ion or molecule to a protein, it is necessary to measure $\bar{\nu}$ and c_A over a wide range of concentration. By then suitably plotting the data one can find values of n and k which, when substituted in equation 1, give a theoretical curve which fits the experimental points. Furthermore, it is possible to learn how many terms are necessary in the right-hand side of equation 1, to achieve a good fit. It is therefore possible to learn how many classes of sites on the protein molecule

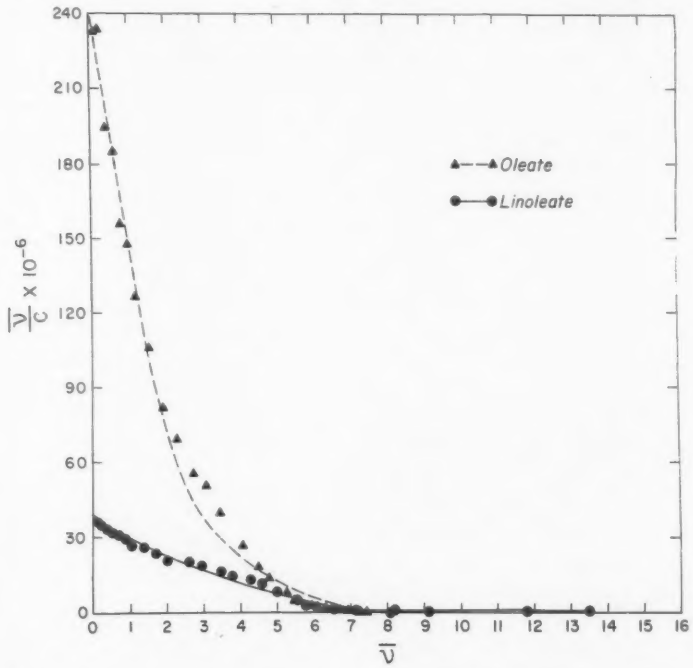

Fig. 7. The interaction of human serum albumin with oleate and linoleate ions. (From GOODMAN, D. S.: *J. Am. Chem. Soc.* 80:3892, 1958.)

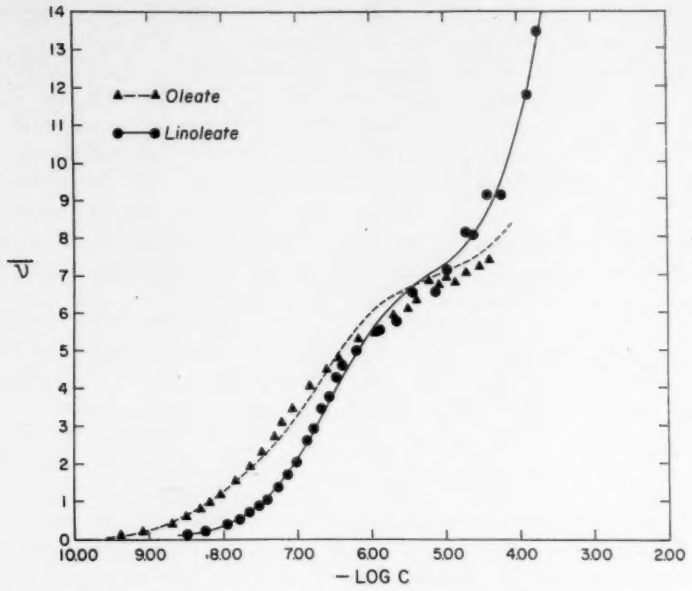

Fig. 8. The interaction of human serum albumin with oleate and linoleate ions. (From GOODMAN, D. S.: *J. Am. Chem. Soc.* 80:3892, 1958.)

are able to bind A, how many sites there are in each class (i.e. the values of n_i), and the values of the association constant for each class of sites (i.e. the values of k'_A).

For the interaction of serum albumin with fatty acid anions, the values of c_A (the unbound fatty acid concentration) were determined as described above. The concentration of bound fatty acid was then determined as the difference between the total fatty acid and the unbound fatty acid concentrations. Dividing the bound fatty acid concentration by the protein concentration gave $\bar{\nu}$ for each experiment.

The results of these studies for the fatty acid ions oleate and linoleate are shown in Figures 7 and 8. These are the two most useful kinds of plots for data of this kind. It should also be pointed out that Figure 8 is of the same form as a familiar titration curve.

Analysis of these data revealed that there are at least three classes of sites on the albumin molecule to which fatty acid ions can be bound. The first class consists of two sites which bind fatty acids extremely tightly. The second class consists of 5 ± 1 sites which bind fatty acids a little less tightly. The third class consists of a larger number, arbitrarily taken to be 20, of weaker binding sites. The values of the association constants for these classes of sites for six fatty acid ions are given in Table VII. The extremely high values of the association constants for the first class of sites (k'_1) show how very tightly two fatty acid ions are bound to albumin.

TABLE VII

The Apparent Association Constants for the Interaction of Human Serum Albumin with Fatty Acid Anions at pH 7.45, Ionic Strength 0.160, 23° C, with Three Classes of Binding Sites, $n_1 = 2$, $n_2 = 5$, and $n_3 = 20$

Fatty acid anion	k'_1	k'_2	k'_3
Laurate	1.6×10^6	2.4×10^5	6×10^2
Myristate	4.0×10^6	1.4×10^6	2×10^3
Palmitate	6.0×10^7	3.0×10^6	1×10^3
Stearate	8.0×10^7	8.0×10^5	1×10^3
Oleate	1.1×10^8	4.0×10^6	1×10^3
Linoleate	1.3×10^7	2.5×10^5	2.5×10^3

(From GOODMAN, D. S.: *J. Am. Chem. Soc.* 80: 3892, 1958.)

The closeness of the fit of the experimental curves constructed with the values of n and k listed in Table VII with the experimental data is shown in Figures 7 and 8. The curves drawn in these figures are all plots of equation 1, using the values of n and k in the table. It is apparent that they fit the experimental points quite well. It should also be mentioned that the values of k in Table VII are called "apparent association constants" because they only apply to these particular conditions (of pH, ionic strength and so on).

Studies have also been conducted on the binding of long-chain fatty acid ions to human low density lipoproteins, and to human erythrocytes.[20,21] These studies reveal that, although both the lipoproteins and the erythrocytes are able to strongly bind fatty acid ions, they are not able to effectively compete with serum albumin until the mole ratio of fatty acid to albumin (i.e. $\bar{\nu}$) is greater than 2. In other words, neither the lipoproteins nor the erythrocytes can compete with the first class of very tight binding sites on albumin. As the mole ratio of fatty acid to albumin is increased further, however, relatively more and more of the fatty acid becomes bound to the lipoproteins (and secondarily to the erythrocytes).

These facts may eventually prove to be of some significance in those clinical conditions which are associated with derangements of lipid levels and metabolism. As previously stated, the normal UFA level in humans is of the order of 0.5 meq/liter of plasma. This level represents a mole ratio of fatty acid to albumin of slightly less than 1. With normal lipoprotein concentrations, less than 2 per cent of the total UFA will be bound to low density lipoproteins. In a condition such as the nephrotic syndrome, however, where albumin concentration is greatly decreased, and lipoprotein concentration is increased, the ratio of UFA to albumin is usually greater than 2, and a much greater proportion of the total UFA will be bound to the lipoproteins. The significance of this is, at present, not known, and is being studied in our laboratory.

Preliminary studies of the composition of the UFA fraction[22] indicate that it is primarily composed of the 16 and 18 carbon fatty acids listed in Table VII. By applying the values of k_1' to equation 1, for a value of $\bar{\nu}$ of approximately 1, we find that the free (unbound) UFA level is of the order of 10^{-8} mole/liter of plasma. In normal human plasma, therefore, less than 0.01 per cent of the UFA present is free in solution. It thus appears that albumin is particularly well constructed to serve as a transport vehicle for UFA. In addition, this raises the interesting question of the mechanism whereby UFA can be rapidly transferred out of the plasma, with so short a half-life, and rapidly transported into cells and oxidized, when its binding to albumin is so very tight. Perhaps there are receptor sites on cell walls which bind fatty acids even more effectively than does albumin, and which can rapidly transfer fatty acids to intracellular loci of oxidation. In any event, although a great deal has been learned about UFA metabolism, it is apparent that there are a great many fundamental questions still to be answered.

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Phospholipids and Blood Coagulation

GEORGE ROUSER, PH.D.*

THE interesting phenomenon of blood coagulation has attracted the attention of physicians and laboratory investigators for many years. For the physician, the problem of hemostasis is a fundamental one since he sees patients in states of hypo- or hyper-coagulability that must be properly diagnosed and managed. The blood clotting mechanism is related not only to thrombosis and hemophilia, but to other diseases of the vascular system such as atherosclerosis.

The clotting mechanism has been found to be complicated and difficult to analyze. These difficulties are due in part to the fact that some of the components in the process are labile and occur in small amounts. Despite these difficulties extensive study has resulted in a progressive increase in our understanding of the basic reactions, and the classic concepts of blood coagulation have been greatly extended. The recent review of Macfarlane[1] makes it apparent that there is a general uniformity of opinion as to the steps in blood clotting. This uniformity may be difficult for the casual reader to appreciate because the various laboratories use different names for the coagulation factors.

THE CLOTTING MECHANISM

Before discussing the role of lipids in coagulation it is necessary to present as a frame of reference the more important findings and the conclusions that are generally accepted regarding the clotting scheme.

As presently visualized, the clotting mechanism may be pictured in part as follows:

$$\text{Prothrombin} \xrightarrow{\text{Prothrombinase}} \text{Thrombin}$$

$$\text{Fibrinogen} \xrightarrow{\text{Thrombin}} \text{Fibrin}$$

The conversion of fibrinogen to fibrin by the proteolytic enzyme thrombin has been studied in considerable detail. It is now thought that thrombin breaks an arginylglycine bond to release acidic peptides from fibrinogen to form fibrin. Fibrin then aggregates to form a clot. The properties of fibrinogen and fibrin and the action of thrombin have been reviewed by Bailey and Bettelheim.[2]

The formation of thrombin from prothrombin is thought to be dependent upon the action of an enzyme here designated as prothrombinase (also referred to as thromboplastin, prothrombin conversion factor, etc.). That this conversion is in fact enzymatic seems very probable, but the proof for this is not entirely conclusive. Although prothrombin has been highly purified, the study of its conversion to thrombin has been hampered by the fact that prothrombinase is poorly defined and is formed as the end-product of a series of reactions. Recent reviews[1,3] have described the present state of our knowledge of the prothrombin to thrombin conversion.

The nature and manner of formation of prothrombinase constitutes the main area of the clotting mechanism in which our knowledge has been greatly extended by recent work. It is thought that there are two different mechanisms for the formation of prothrombinase, the intrinsic and the extrinsic. The intrinsic system requires only blood components, whereas the extrinsic system depends upon material derived from tissues as well. The concept of two different mechanisms has done much to clear up confusion in this area. Both systems may be involved in physiologic hemostasis since patients with deficiencies in either one or

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the other system have been observed to bleed in an abnormal fashion.[4–7] The factors currently thought to be involved in the formation of prothrombinase in the two systems are:

Intrinsic	*Extrinsic*
1. Platelets	1. Thromboplastin (a particulate lipoprotein preparation obtained from tissues)
2. Antihemophiliac globulin (AHG)	2. Proconvertin (Factor VII)
3. Plasma thromboplastin component (PTC), or Christmas factor	3. Calcium
4. Calcium	4. Proaccelerin (Factor V)
5. Proaccelerin (Factor V)	

The extrinsic system has been analyzed very carefully in Owren's laboratory and a recent summary of the findings of this school has been presented in the form of a lucid monograph by Hjort.[4] The extrinsic system is thought to be initiated by tissue damage with the release of tissue thromboplastin. Tissue thromboplastin is particulate and can be prepared by saline extraction of brain or other tissues. Tissue thromboplastin has been purified and studied extensively by Chargaff and his co-workers[8] who have found it to be a high molecular weight lipoprotein particle.

Tissue thromboplastin in the presence of calcium reacts with proconvertin (a protein) to form the complex termed convertin. The entire complex is then thought to react with accelerin (a protein), formed from a precursor, proaccelerin, in the presence of calcium, to give the enzyme prothrombinase. Several difficulties, discussed in detail by Hjort,[4] are still present with regard to the complete analysis of this system.

The intrinsic prothrombinase generating system requires some factors that do not participate in the extrinsic system. The initial event in the intrinsic system (which requires only products present in the blood) is believed to be contact with a foreign surface, as perhaps by a break in the vascular endothelium. Then, it is believed that plasma thromboplastin component (PTC or Christmas factor) is activated and reacts with antihemophiliac globulin (AHG), calcium, and platelets to give an intermediate product which reacts with accelerin to yield prothrombinase.[3] It is quite probable that still other factors enter into the reaction sequence.[9]

LIPID PARTICIPATION IN COAGULATION

It has been thought for many years that lipids are involved in coagulation. In 1886, Wooldridge[10] suggested that a lipoprotein might be involved in the coagulation of blood, and in 1912 Howell[11] presented evidence which indicated that the cephalin fraction from tissues contained the active substance. Since that time, however, lecithin, phosphatidyl ethanolamine, phosphatidyl serine, inositol phosphatides, and fatty acids have been reported as active substances. All investigators have agreed, however, that lipids can make plasma clot more rapidly. It was demonstrated very early that tissue thromboplastin was completely inactive if lipids were removed by treatment with organic solvents, and at least a part of the activity could be regained by recombination of the lipid and protein.[12–15] It has been long felt that an active principle in blood platelets is lipid in nature, and the observations of Macfarlane *et al.*[16] on the incoagulability of plasma from which lipids have been removed by extraction, makes it appear that the intrinsic system, as well as the extrinsic system, requires lipid material for coagulation to take place.

NATURE OF THE THROMBOPLASTIC PHOSPHATIDE

In our laboratory, interest in the problem of the nature of the lipid material that accelerates coagulation has stemmed from two basic facts. First, this activity, alone among those that have been attributed to phosphatides, offered the possibility for a detailed study of lipid structure as related to an important biologic activity. Second, and potentially of great clinical significance, is the fact that lipid preparations have been shown to be capable of replacing, at least in part, the functions of the blood platelets. White *et al.*[17] demonstrated that dogs made thrombocytopenic by x-irradiation showed *in vitro* evidence of a reduced bleeding tendency after intravenous infusion of a mixed soybean phosphatide preparation. Stefanini and Campbell,[18] Hayhoe and Whitby,[19] White,[20] Rosen *et al.*,[21] and Steven-

son[22] have observed improvement in the hemostatic mechanism or *in vitro* clotting tests in platelet deficient humans after the infusion of phospholipid preparations from platelets, brain, or soybean. Thus, it is evident that the separation and chemical characterization of the active thromboplastic substance may serve a useful clinical function.

Because we hoped that our laboratory findings would serve as a guide to subsequent clinical work, we have used fresh plasma obtained by technics designed to minimize platelet fragmentation and *in vitro* changes in plasma clotting factors.

Methods: Blood was drawn by two technics. When samples were to be obtained from a blood donor prior to the collection of a unit of blood for transfusion purposes, the blood was allowed to pass through a plastic tube and to drip into siliconed test tubes. The first 5 to 10 ml of blood was discarded and the sample was then collected using one volume of 3.12 per cent sodium citrate to nine volumes of blood. When a sample was to be withdrawn from laboratory personnel, a two syringe technic was used. Blood in the first syringe was discarded, and the sample was collected in the second syringe previously lubricated with silicone oil (General Electric SF-96). Centrifugation was begun no more than 20 minutes after venipuncture, and was carried out at 7,000 × g for 30 minutes in a refrigerated centrifuge. The upper two-thirds of the low-platelet plasma was removed and kept in siliconed tubes in an ice bath. Occasional platelet counts performed by phase contrast microscopy on these plasma preparations revealed less than 10^4 platelets/mm^3. Plasma was used only on the day of collection whenever active lipids were to be compared. Preliminary tests were performed in some cases with plasma that had been stored in the frozen state.

The clotting tests were performed as follows: a 0.2 ml aliquot of plasma was transferred by means of a siliconed pipette to a nonsiliconed test tube to which a 0.2 ml volume of veronal buffer (sodium diethyl barbiturate, 11.75 g; sodium chloride, 14.67 g; 0.1 N hydrochloric acid, 430 ml; water to 2 liters), or 0.2 ml of the lipid fraction dispersed in veronal buffer was then added, and the mixture was incubated for three minutes (by stopwatch) prior to the addition of 0.2 ml of 0.025 M calcium chloride. The end point was the time required for the formation of the first fibrin threads and was recorded in seconds from the time of recalcification. All values obtained in the test were averages of at least five determinations at each concentration of lipid tested.

It is extremely important to realize that control clotting times and the ability of an active phosphatide preparation to shorten the clotting time may be different for different plasma samples taken from apparently healthy individuals. Thus, it is necessary to compare active substances using a single plasma sample. The most reliable way to determine the relative activity of two active phosphatides is to determine clotting times with the two preparations run at the same time with the same plasma sample (see Table I).

Results and Discussion: Using the technics just described the following major points were established:[23,24]

(1) Only phospholipid can appreciably shorten the recalcification time.

(2) The specific phospholipid found to accelerate clotting was phosphatidyl ethanolamine.

(3) Phosphatidyl ethanolamines isolated from various natural sources were found to accelerate clotting to different extents. This was shown to be related to the fatty acid composition of the phosphatides.

(4) The ability to shorten the clotting time was observed to increase as the degree of unsaturation of the fatty acids in the molecule increased. Maximum activity was found for the phosphatidyl ethanolamines in which both of the fatty acids were unsaturated. Phosphatidyl ethanolamines containing one saturated and one unsaturated fatty acid were not as effective.

(5) When proper precautions were taken against oxidative degradation of the phosphatides, it was possible to isolate all of the initial activity of a phosphatide mixture in the phosphatidyl ethanolamine fraction. Previous failures to recover activity quantitatively were probably due to oxidation of phosphatide fractions during separation.

(6) The necessity for unsaturation in the fatty acids was confirmed by hydroxylation with performic acid. This procedure destroys double bonds and produces a more nearly water soluble product that has little or no clot accelerating activity.

(7) The necessity for the presence of the free amino group was demonstrated by removal of this group by reaction with ninhydrin and by acylation with acetic anhydride. Phosphatidyl ethanolamine altered by reaction with these reagents had little or no clot-accelerating activity.

(8) The greater activity associated with the greater degree of unsaturation of symmetrically distributed fatty acids of the phosphatide was postulated to be the result of the formation of smaller micelles as unsaturation increased. The smaller micelles would have a greater surface for reaction, with a consequent increase in reaction rate.

Since our initial publications, we have reexamined the activity of phosphatidic acids. Phosphatidic acids, produced by degradation of lecithins or phosphatidyl ethanolamines with the plant enzyme phospholipase C, when originally examined were found to have some ability to accelerate clotting. No phosphatidyl ethanolamine was detected in these preparations by paper chromatography, and when dipalmitoleyl lecithin was used as substrate in the enzyme reaction it was felt that a phosphatidic acid was produced with true activity. These studies were repeated using the carrot enzyme extract prepared exactly as described by Kates[25] and the incubation conditions and recovery of the phosphatidic acid as reported by Kates.[26] Phosphatidic acid prepared using dipalmitoleyl lecithin as substrate was found to be a slight inhibitor of clotting. The previous finding of clot-accelerating activity in a similar preparation was probably due to the presence of a small amount of phosphatidyl ethanolamine extracted from the very crude and less active enzyme preparation from cabbage. These findings illustrate one of the basic problems in assigning biologic activity to a lipid fraction. A very small contamination that may go undetected by very sensitive methods may be mistaken for activity of the major phosphatide component of a mixture. To avoid this difficulty with phospholipid fractions to be tested for clot accelerating activity, it can now be recommended that the fractions be treated with ninhydrin to destroy traces of phosphatidyl ethanolamine.

O'Brien[27] has reported that liver inositol phosphatide is active in the recalcified plasma system. A sample of liver inositol phosphatide, presumably of similar structure, was found in our laboratory to be a slight inhibitor of coagulation. It is possible that O'Brien's preparation contained some phosphatidyl ethanolamine and, if so, treatment with ninhydrin would destroy the activity of the preparation. It has been observed that treatment of mixed phosphatide preparations with ninhydrin destroys most of the thromboplastic activity.[21] The clot accelerating activity of brain diphosphoinositide reported by O'Brien[27] may have been due to the presence of phosphatidyl ethanolamine since Overman and Wright[28] have reported that both brain and soybean inositol phosphatide preparations inhibit coagulation. The variable clot accelerating activity of phosphatidyl serine preparations obtained from different laboratories that was reported by O'Brien[27] may well have been due to the presence of different amounts of phosphatidyl ethanolamine. Unfortunately, no simple reaction such as ninhydrin treatment is available for the destruction of phosphatidyl ethanolamine in phosphatidyl serine preparations. The precipitation technic described by Silver *et al.*[29] can be used to obtain phosphatidyl serine preparations that prolong the *in vitro* clotting time of the extrinsic clotting system (tissue thromboplastin added) and are *in vivo* anticoagulants. The results of Silver *et al.* are in keeping with the findings of Mushett *et al.*[30] and Goldsmith and Mushett.[31] It seems most probable at this time that the intrinsic and extrinsic clotting systems are inhibited by pure, well-dispersed acidic phospholipids such as phosphatidyl serine, inositol phosphatides, and phosphatidic acids and that clot accelerating activity in the intrinsic system is due to phosphatidyl ethanolamine.

In contrast to the highly specific requirement for phosphatidyl ethanolamine with the proper

fatty acid composition in the intrinsic clotting system, the test system employing Russell viper venom where venom is thought to replace tissue thromboplastin is relatively nonspecific.[23,27] A brief report by Kuhn and Klesse[15] indicates that the extrinsic system may be relatively nonspecific like the venom system, but more detailed investigation is required to establish the structural requirements of the system.

Confirmation of our previous finding that fully saturated phosphatidyl ethanolamines possess only slight clot accelerating activity has been obtained by hydrogenation of several natural phosphatide preparations. More important new data, however, have been obtained with a new synthetic phosphatidyl ethanolamine. Dr. Eric Baer has recently completed the synthesis of diolelylphosphatidyl ethanolamine and has generously supplied us with one of his preparations only a few days old. The white, waxy solid was difficult to disperse in buffer, but sonication for ten minutes with a 10 kilocycle Raytheon sonic oscillator (250 w) yielded a well-dispersed preparation that slowly formed coarse visible aggregates on standing (30–60 minutes from time of sonication). The formation of coarse visible aggregates could be prevented by the addition of a small amount of oleic acid or a dipalmitoleyl lecithin preparation that contained some free fatty acid. The clot accelerating activity was not changed by these additions, however. The best dispersions of this preparation of dioleylphosphatidyl ethanolamine were compared to soybean phosphatidyl ethanolamine in the recalcified plasma system described above. Soybean phosphatidyl ethanolamine was shown previously to be largely dilinoleylphosphatidyl ethanolamine. The soybean product was expected to be more active than the synthetic dioleyl product, since it contains fatty acids of greater degree of unsaturation (average of two double bonds per molecule of fatty acid compared to one per molecule for the dioleyl product). Table I shows that, as expected, the soybean preparation is more active. It can be seen that for a given weight of soybean phosphatidyl ethanolamine, approximately 20 times the weight of the dioleyl compound is necessary to produce the same clotting time. Similar results were obtained using different low platelet plasma samples.

TABLE I

Relative Activity of Dioleyl and Soybean Phosphatidyl Ethanolamines in Recalcified Plasma

Dioleyl		Soybean	
μg/ml	Clotting time in sec	μg/ml	Clotting time in sec
500	104	25	103
200	122	10	120

These new data with the unsaturated synthetic phosphatidyl ethanolamine are in keeping with two major conclusions from our previous work: (1) that phosphatidyl ethanolamine is active, and (2) that activity increases with increase in unsaturation of symmetrically distributed fatty acids. It is to be noted that synthetic dioleyl phosphatidyl ethanolamine is more active than synthetic distearoyl or dimyristoyl phosphatidyl ethanolamines.

PHOSPHATIDYL ETHANOLAMINE IN PLASMA

Since phosphatidyl ethanolamine is the only lipid that will greatly accelerate clotting of the intrinsic system, it becomes important to determine whether this substance is necessary for clotting to take place by the intrinsic mechanism. This question is important because it may be related to the mechanism by which blood is normally maintained in the fluid state *in vivo* and to the process whereby blood becomes hypercoagulable. Hypercoagulability and thrombosis are thought by some to be related to both blood lipid levels and atherosclerosis.

The platelets are generally assumed to provide a lipid factor for coagulation. *In vitro*, phospholipid is at least as active as platelets are in shortening plasma clotting times.[27] Furthermore, the most active phosphatidyl ethanolamine encountered in our studies was obtained from human blood platelets.[23,24] The data are thus in keeping with the common idea that platelets contribute a necessary lipid cofactor for normal coagulation.

If one role of platelets is to furnish a lipid cofactor, it would be expected that plasma

would contain very little phosphatidyl ethanolamine, or that this lipid would be bound in some inactive form. It appears that plasma contains, at most, only small amounts of phosphatidyl ethanolamine. Taurog *et al.*,[32] Sinclair,[33,34] and Hack[35] have reported that plasma phospholipid is virtually all of the choline-containing variety in the dog and man. Results in our own laboratory have indicated that normal human plasma contains, at best, only traces of phosphatidyl ethanolamine (1 to 2 mg/100 ml of blood). These results were obtained by direct isolation of the phospholipids followed by chromatographic examination. By an indirect method Axelrod and associates[36] have determined that phosphatidyl ethanolamine constitutes 3 per cent of human plasma phospholipids. It must be realized that these small amounts of phosphatidyl ethanolamine could have been contributed by the rupture of platelets or leukocytes (also rich in phosphatidyl ethanolamine) brought about by *in vitro* manipulation. It is particularly important to realize that a few platelets can still be detected in plasma after high speed centrifugation and that some of these fragile elements may have been disrupted despite the precautions taken to minimize damage.

FAT INGESTION, HYPERCOAGULABILITY, AND ATHEROSCLEROSIS

The hypothesis has been advanced that ingestion of a fatty meal leads to an increased coagulability of the blood, and that this may be related to an increased tendency to thrombosis which may be an important factor in atherosclerosis. The data relevant to these relationships have recently been discussed in some detail by O'Brien.[37] It appears that some, but not all, investigators have observed a decreased coagulation time with whole blood in siliconed vessels that is not observed in glass. The meaning of such findings is not clear. It can be said only that there may well be an increased coagulability of the blood after a fatty meal, but that the data presently available are inadequate to establish conclusively such an increase.

It has been suggested that an increase of unesterified fatty acids in plasma could produce a hypercoagulable state after a fat meal. Although some fatty acids added to plasma *in vitro* do shorten the clotting time to a measurable extent,[23,27] the observation that the higher fatty acids are transported in the blood tightly bound to albumin[38] makes their participation in clotting reactions seem improbable.

Chylomicra have been reported to accelerate clotting, but this effect has been reported to be slight.[37] Since chylomicra contain phospholipid, it is reasonable to suppose that they may contain some phosphatidyl ethanolamine. Robinson and Poole[39] have presented data indicating the presence of phosphatidyl ethanolamine in chylomicra, but no definitive study on this problem has been reported to date.

It can be concluded that a significant increase in the *in vivo* coagulability of the blood after a fat meal has not been demonstrated with certainty, nor has the presence of an increased amount of a lipid substance in an available form that can promote clotting been demonstrated after fat feeding. It must be realized, however, that the methods for study are crude and that no reliable technic for the evaluation of the *in vivo* status of the coagulability of the blood is available. The relationship between fat feeding, coagulation, and atherosclerosis must be investigated more extensively.

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Nutrition News

"1958 Bibliography of Medical Reviews" Available

Announcement is made of the publication of the 1958, 3rd annual, volume of the *Bibliography of Medical Reviews*. This publication is arranged by subject with a separate author index and will contain approximately 2,900 references to review articles in clinical and experimental medicine and allied fields which have appeared largely in 1957. Copies of Volume 3 for 1958 will be available from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.

Fellowships for Research in Clinical Nutrition

To stimulate staff members and students of schools of medicine to take a more active interest in the science of nutrition, the Nutrition Foundation, Inc., in cooperation with the Council on Foods and Nutrition of the American Medical Association, is sponsoring ten research fellowships in clinical nutrition. These fellowships are given in honor of Dr. Virgil P. Sydenstricker, recipient of the 1958 Joseph Goldberger Award in Clinical Nutrition.

The stipend will be $200.00 per month for three months.

The conditions of the fellowships are: (1) the grant shall be made to a medical student upon recommendation of a senior investigator; (2) the recipient will be selected by the Council; (3) the grant shall be used solely for remuneration of the selected medical student; (4) the grant shall be limited to a period not to exceed three months during the nonacademic portion of school year; (5) no senior investigator may have more than one student per year receiving a grant; (6) only a brief progress report or summary will be required, and publication will be at the discretion of the senior investigator.

Application is to be made in writing by the senior investigator to: Council on Foods and Nutrition, American Medical Association, 535 North Dearborn Street, Chicago 10, Ill., on or before December 15, 1958. The application shall include: (a) a brief outline of study proposed; (b) assurance that adequate physical facilities are available; (c) assurance (but not necessarily identification) of the availability of a qualified medical student.

Nutrition Education Programs

The National Vitamin Foundation, in active cooperation with the Commissions on Nutrition of the Pennsylvania State Medical Society and the Philadelphia County Medical Society, is developing nutrition education programs for the practicing physicians. It is planned to present at the monthly meetings of the various county medical societies a discussion of the basic and practical aspects of nutrition as they relate to disease. The following programs have been scheduled for late 1958 and early 1959:

Date	*Speaker and subject*	*Place*	*Medical society*
December 3, 1958	RICHARD VILTER, M.D.: Nutritional and Metabolic Aspects of Cardiovascular Disease	Lancaster, Pa.	Lancaster
January 10, 1959	NORMAN JOLLIFFE, M.D.: Fats, Cholesterol, and Atherosclerosis: A progress report	Atlantic City, N. J.	New Jersey Academy of General Practice

Reviews of Recent Books

Principles of Research in Biology and Medicine, by Dwight J. Ingle. Lippincott, Philadelphia, 1958, pp. 120, $4.75.

In this little book a wise scientist offers the fruits of many years of reflection on the art and science of Research itself. A humanitarian as well as a top-notch investigator, Dr. Ingle has written in a simple style on such complex subjects as the methods of science and its basic assumptions, the limitations of mind, causality, probability, experimental errors, clinical investigation and the nature of creativity. But in many pages one can glimpse the author's concern for the individual. This is noted especially in the last chapter on Relationships Among Scientists. One gathers that the writer thoroughly enjoys his work and at the same time has immense reverence for his calling.

A notable characteristic of the book is the profusion of appropriately amusing quotations from *Alice in Wonderland*. If there is any criticism of this book, it is that it is too brief: in a number of instances one would have wished for a fuller discussion. However, as indicated in the preface, the book is chiefly intended for students who are preparing for or are beginning research in biology and medicine. They will certainly profit from it. So will many others in research who perhaps consider they know "all about it." This reviewer believes every reader will profit to some extent by reading this interesting book. S. O. W.

Metabolic Regulation, by Théophile Cahn. Editions Presses Universitaires, Paris, 1956, pp. 70.

This book by the Director of Research of the CNRS (Centre National de Recherches Scientifiques), will be of greatest value for all institutions where the physiology of nutrition, and particularly the physiology of the intermediary metabolism, is studied.

As newer technics continuously enhance our knowledge, and more and more separate specialities develop, it becomes increasingly difficult to retain an overall view of the essential problems. The author has attempted to present as complete a synthesis as possible of all earlier studies concerning metabolic regulations. The book is therefore a valuable reference work with much valuable data which previously were scattered throughout many monographs and journals.

In a preliminary chapter on diabetes mellitus, one of the major subjects of physiologic study, a review is presented of the studies in this field and of the evolution of the concepts which have led to the present perfected methods and satisfactory results. Then follows the first and the most important part which deals with the circulation of metabolites in the organism.

Glycemia is elucidated from different points of view: constancy, provoked hyperglycemia, renal threshold, peripheral and hepatic glycemia. Subsequently, the author discusses the influence of the nerve centers and especially of the endocrine glands: the pancreas and its effect on the blood sugar level. A whole chapter is devoted to the physiology of insulin, also, the reciprocal influences of the hypophysis, the adrenals and the thyroid, and finally the blood sugar levels of animals with alloxan and with phlorhizin diabetes.

Another chapter deals with the role of the liver in carbohydrate metabolism: glycogenolysis and glycogenesis, *in vitro*, hepatic glycoregulation *in vivo*, and the influence of the endocrine glands on this process.

The author subsequently discusses the circulation of the lipids under hormonal influences, and the role of the liver and of the muscles in lipid metabolism. A more concise discussion is devoted to the proteins insofar as they can be regarded as metabolites playing a part in the process of energy production. The problem of the circulation of water and ions is studied here only in regard to the relations existing between their metabolism and the endocrine glands.

The value of this book, which will doubtless be of great service to the profession, is enhanced by an extensive bibliography. Even if the scope of the subjects dealt with does not permit completeness, the book gives, in 70 pages, the essentials of the subject.

HUGUES GOUNELLE

Drugs—Their Nature, Action and Use, by Harry Beckman. Saunders, Philadelphia, 1958, pp. 728, $15.00.

The prolific professor of pharmacology, Dr. Harry Beckman, has written an unusual textbook for medical students. It has the remarkable virtue of being an exceptionally readable students' text at the same time that it is a good reference text for practicing physicians.

The advantages of this book may be listed as follows: The style is refreshingly simple, clear, and has the personal touches characteristic of the author's other works. Much attention is paid to the recently introduced drugs (and reference to common trade names is helpful). There is a copious index. The typography is pleasant

and use of bold-face subheads is a useful feature in such a text.

These virtues often contain within themselves certain potential defects, which should be pointed out for the reader to evaluate. The admirable, almost chatty, style tends to be exceptionally persuasive. And while every author has the right to express opinions, this may raise interesting questions such as: Is it "right" for medical students to be exposed (and likely convinced thereby) to strong opinions when they are not yet in a position to evaluate all the statements? Related to this is the frequent use of *one* brand name as an example of a class of compounds. Students might believe, for example, that Hyflavin is the name of the only riboflavin preparation. Certainly all brand names for all drugs could not, and need not, be listed. The problem is difficult and not easily solved.

Similarly, discussing six synthetic belladonna-substitutes in the treatment of Parkinsonism would seem to be more useful to the prescribing physician than to the medical student who perhaps has not yet seen a patient with the disease.

These comments are not criticisms of Beckman's book but rather point out the problem of a pharmacologic text prepared for the student. Certainly this text can be highly recommended and will be deservingly popular with medical students and practitioners alike. S. O. W.

Clinical Enzymology, ed. by G. J. Martin, Little, Brown, Boston, 1958, pp. 241, $6.00.

Fundamentals deemed essential to understanding medical applications of enzymology are presented in this relatively small volume. Only two of the seven chapters actually elaborate upon current clinical enzymology (parenteral and diagnostic uses, respectively). The editor is responsible for three chapters which are indirectly clinical. Provocative speculation and astute review of selected experimental data form the matrix of this text. Physical chemistry of enzymes is discussed generally by M. J. Sullivan. Pharmacological and classical biochemical aspects are discussed by J. M. Beiler. Parenteral applications are covered in 34 pages by H. Tanyol, W. M. Swain, and J. M. Beiler. Clinical pathology and diagnostic uses of enzymes are summarized in 47 pages by G. V. Rossi.

In the clinical chapters, the information appears to be more suggestive than conclusive. Diagnostic and prognostic value of tissue and blood levels of various enzymes are discussed. Proper caution against "precision diagnosis" on the basis of specific enzyme levels is stressed, in view of fluctuations in activity of particular enzymes. Limitations of past experimental studies with impure enzymes are discussed. For the average clinician, the chapters on protein biology, polymerases, and perspectives may be frustrating. However, the biologist, physiologist, and pharmacologist will be intrigued by the keen review of facts and the enthusiastic manner of presentation. Analogies are made between the antigen-antibody relationship and enzymatic activity, centered about the complementariness of the respective interacting molecules. Three enzymes are emphasized as of potential application in arteriosclerosis, collagen diseases, and cancer-elastase, collagenase and ribonuclease, respectively.

The text is an intellectual shelf-mate to the recent (1957) Vol. 68, Art. 1, p. 244, *Ann. New York Acad. Sc.* monograph on the clinical applications of proteolytic enzymes, for which G. J. Martin was consulting editor. In the words of the editor of the text, clinical enzymology is dedicated, at least, . . .secondarily. . .to the proposition that restoration of normal enzymatic milieu is essential to a return to health and that a significant directional force toward that restoration can be achieved by the administration of enzymes." E. COHEN

Health Yearbook 1957, compiled by Oliver E. Byrd, Stanford Univ. Press, Stanford, Calif., 1958, pp. 278, $5.50.

Those concerned with the health problem in its broad psycho-socio and physical aspects have undoubtedly found this annual Health Yearbook of sufficient value to encourage the editor and publishers through 15 years of research, selection, and publication. For this 15th edition, as in the previous editions, approximately 2,000 articles were screened, and about 250 selected for abstracting.

The readers of this JOURNAL will of course be particularly interested in the 14-page chapter on Nutrition and Health which is subdivided into sections under the following headings: Social and Cultural Implications of Food and Food Habits; The Meaning of Food; Fats in thd Diet; Fats and Heart Disease; Diet and Heart Disease; Dietary Fat and Deaths from Heart Disease; Cancer and Food; Food and Goiter; Food Allergy and Diarrhea; Coffee and Obesity; Vitamin D Intoxication; Nicotinic Acid Overdosage; Nail Defects and Gelatin; Food Faddists Badly Nourished.

One may, however, question the adequacy of so brief a chapter covering so vast and important a literature. In this JOURNAL, as an example, during 1957 approximately 75 original articles were published plus abstracts of over 300 articles which had been published in other specialty, general medical, and scientific journals.

In format, the book is well set up, including a good bibliography and subject and author index. C. I. P.

Chemistry of Lipides as Related to Atherosclerosis, ed. by I. H. Page, Thomas, Springfield, Ill., 1958, pp. 334, $8.50.

In May, 1957, a symposium attended by 95 outstanding scientists was held under the auspices of the National Advisory Heart Council. The presentations and discussions which followed form the substance of this book. Among the authors and discussers are investigators in universities, governmental institutions,

and industry. As the title suggests, emphasis is on biochemistry of lipids. Problems in lipid transport, metabolism, and biochemical interactions are described for the various lipid fractions. All the papers are well written and clearly illustrated. The most clinical papers are by the Rockefeller group, which present much of the material concurrently presented in medical journals. As is often found in proceedings of symposia, the discussions after the papers are, occasionally, even more illuminating than the articles themselves. Throughout this book the presentations are of a uniformly high level and the text shows, here and there, the hand of the able editor.

This book should appeal to all investigators in the field of atherosclerosis as well as in lipid metabolism. It presents some of the best views of this important aspect of nutrition. S. O. W.

Office Gastroenterology, by Albert F. R. Andresen. Saunders, Philadelphia, 1958, pp. 707, $14.00.

Dr. Andresen has courageously essayed to write an up-to-date text covering all features of gastroenterology in a single volume. He writes from a wide experience and has attempted to cover all possible subjects, clinical entities, technics, and clinical problems likely to be faced in the treatment of digestive diseases. Despite its almost encyclopedic completeness, the monograph is well arranged and reads easily, and provides a valuable reference volume.

Dr. Andresen's strong opinions about the importance of gastrointestinal allergy are set forth at length, including instructions on the allergic therapy for chronic ulcerative colitis. In view of his own extensive experience in this area, one can pardon his failure to indicate the highly controversial nature of the subject matter presented. There are other areas of treatment in which Dr. Andresen takes what may be considered a controversial or possibly minority position. For instance, he outlines a treatment for massive gastrointestinal hemorrhage, indicating what would be considered by most practicing gastroenterologists today as an inadequate blood transfusion program. In describing the treatment of Laennec's cirrhosis associated with ascites, Dr. Andresen is less emphatic than most of his colleagues in his instructions regarding salt restriction. Although other details in the management of chronic ulcerative colitis require pages, he dismisses steroid hormone therapy in a sentence.

The book is well-indexed; it does, however, have a lack of bibliographic references. The book suffers from the nature of the specialty of gastroenterology itself. To present all aspects of the field in one volume requires a brevity which makes it difficult or impossible to be sufficiently complete. Therefore, this one-volume work is more suitable for the internist than for the gastroenterologist. JAMES B. HAMMOND

Chemistry and Biology of Mucopolysaccharides (Ciba Foundation Symposium), edited by G. E. W. Wolstenholme and M. O'Connor, Little, Brown, Boston, 1958, pp. 329, $8.50.

The rapid expansion of knowledge of carbohydrate-protein complexes is well highlighted by this Ciba Symposium on the chemistry and biology of mucopolysaccharides. Since the symposium was not restricted to a precise definition of "mucopolysaccharide," the participants, ranging from sugar chemists to clinical pathologists, were able to present a broad group of biochemical, biologic, and immunologic studies of sialic acid, neuraminic acid, glycoproteins, mucoproteins, orosomucoid, polysaccharides, and related substances. This has resulted in an impressive and interesting biochemical panorama with comprehensive chapters on blood group specificity by Morgan, serum glycoproteins by Winzler, urinary mucopolysaccharides by Maclagan, bacterial polysaccharides by Kabat, Dorfman, Westphal, and others, and the role of mucoprotein in the virus hemagglutination reaction by Gottschalk. A wide variety of chemical degradation and analytical studies on plant, bacterial, and animal mucopolysaccharides (even including those from human milk) are also presented and critically discussed by Dische, György, Stacey, and others. Workers in various fields will welcome this broad coverage, since it emphasizes the many common chemical problems which exist in the analysis of the complex and unusually tight chemical bonds between protein, hexoses, hexosamines, and the recently delineated sialic acid-neuraminic acid moieties. The symposium amply affirms the major advances which have been made in establishing the chemical composition and physical characteristics of these ubiquitous carbohydrate-amino-sugar-protein substances from bacteria, serum, spinal fluid, connective tissues, etc. Kabat's brilliant attempt to correlate bacterial polysaccharide structure with immunologic reactivity as determined by oligosaccharide side chains is particularly well presented in this publication.

While the importance of glycoproteins as general biologic building blocks is evident from this comprehensive publication, one might have wished for a relatively greater physiologic emphasis on some of the presentations. The pathways involved in biosynthesis, breakdown, and turnover of these mucopolysaccharide substances *in vivo* remain obscure. The reader looking for an answer to the question of their immediate physiologic significance for pathologists and clinicians will not find it here. This merely reflects the status of our understanding of these substances.

It is unfortunate that the symposium did not conclude with a résumé in which each participant could discuss the possible implications of his work for future physiologic investigators. Such a chapter could have reviewed the fragmentary bits of evidence which hint at the possible function of serum mucoprotein complexes

(seromucoid, orosomucoid) along enzymatic, immunologic, serologic, coagulation, or even hormonal lines. The most invaluable portion of this book is the highly stimulating, and often extended discussions by the many eminent contributors following each paper. These contributions emphasize the necessary preoccupation with biochemical methodology which still pervades most researches on mucopolysaccharides.

EZRA M. GREENSPAN

Annual Review of Medicine, vol. 9, edited by D. A. Rytand, Annual Reviews, Inc., Palo Alto, 1958, pp. 488, $7.00.

The 1958 volume of the *Annual Review of Medicine* contains, in addition to its ample coverage of an expanding literature, a fine chapter on nutrition and nutritional deficiency diseases by Vilter and Will. Special mention should also be made of an interesting chapter by Lasagna and Meier on the clinical evaluation of drugs, of two chapters on laboratory aids (paper chromatography and enzymology) and one on Soviet medical research.

As usual, several thousand references are briefly mentioned and woven into a running text which points out the highlights of current investigation as seen by the authoritative authors. This book again merits praise as a useful guide to the serious investigator in almost every field of medicine.

S. O. W.

A Primer for Coronary Patients, R. J. Needles. and E. M. Stoney. Appleton-Century-Crofts, New York, 1958, pp. 176, $3.75.

This volume represents the successful joint effort of a physician and a writer to explain to the layman what is known about coronary heart disease; the nature and manifestations of the disease, the complications, the treatment and prevention.

The book is well conceived and well written and the subject is dealt with thoroughly and accurately. The authors are to be complimented on the extent to which they have succeeded in writing from the point of view of the patient or his friend or relative, so that there is hardly a question that might come up that is not answered. All medical terms are carefully explained or paraphrased (with the exception of "hiatus hernia" on page 31). A number of illustrations add to the clarity of the text.

The point of view represented is conservative, stressing that much is known about coronary disease, and that the well-informed patient can live a satisfying life notwithstanding his illness and can greatly improve his prognosis.

This book may be highly recommended to the intelligent patient with coronary disease and his family.

W. H. ABELMANN

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

The Guinea Pig in Research. Biology-Nutrition-Physiology by Mary Elizabeth Reid, Human Factors Research Bureau, Washington, D. C., 1958, pp. 87, $2.00.

Cholesterol by David Kritchevsky, Wiley, New York, 1958, pp. 291, $9.75.

Thirst. Physiology of the Urge to Drink and Problems of Water Lack by A. V. Wolf, Thomas, Springfield, Ill., 1958, pp. 536, $12.50.

Remedies and Rackets. The Truth About Patent Medicines Today by James Cook, W. W. Norton, New York, 1958, pp. 252, $3.75.

Advances in Clinical Chemistry, Volume I, edited by Harry Sobotka and C. P. Stewart, Academic Press, New York, 1958, pp. 398, $12.00..

Medicine and the Navy 1200–1900, Volume II 1619–1714 by J. J. Keevil, E. & S. Livingstone, Edinburgh, 1958 (Williams and Wilkins, American agents), pp. 332, $8.50.

Ciba Foundation Colloquia on Ageing. Volume 4. Water and Electrolyte Metabolism in Relation to Age and Sex, edited by G. E. W. Wolstenholme and Cecilia M. O'Connor, Little, Brown, Boston, 1958, pp. 327, $8.50.

Ciba Foundation Symposium on the Neurological Basis. of Behaviour, edited by G. E. W. Wolstenholme and Cecilia O'Connor, Little, Brown, Boston, 1958, pp. 400, $9.00.

Food Habits and Nutrient Intakes in a Siamese Village. Studies in Bang Chan 1952–1954 by Hazel M. Hauck, S. Sudsaneh and Jane R. Hanks, Cornell University, Ithaca, N. Y., 1958, pp. 129, $1.00.

The Interference Microscope in Biological Research by Arthur J. Hale, E. & S. Livingstone, Edinburgh, 1958 (Williams and Wilkins, American agents), pp. 114, $5.00.

Deficiency Disease. Functional and Structural Changes in Mammalia which Result from Exogenous or Endogenous Lack of One or More Essential Nutrients by Richard H. Follis, Jr., Thomas, Springfield, Ill., 1958, pp. 577, $14.75.

Food and You by Edmund Sigurd Nasset, Barnes and Noble, New York, 1958, pp. 166, $1.25.

Abstracts of Current Literature

CHARLES R. SHUMAN, M.D., EDITOR

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DIETARY FATS AND SERUM LIPOPROTEINS

Rapidly accumulating evidence indicates that the neutral fats and unesterified fatty acids are the dynamic metabolites of the lipid series in the utilization of fats. The previous concept that phospholipid is the most readily utilizable form for fat metabolism is discarded. The use of fat in tissue metabolism may follow the scheme of (1) mobilization of depot fat, (2) release of free fatty acids, (3) rapid oxidation of free fatty acids, the initial phases being dependent upon the activation of tissue and serum lipolytic lipases. Elevation of the free fatty acids is associated with fasting or epinephrine administration and depression with feeding, glucose, or insulin administration.

Unesterified Fatty Acid in Human Blood Plasma. II. The Transport Function of Unesterified Fatty Acid. R. S. Gordon, A. Cherkes, and H. Gates. *J. Clin. Investigation* 36: 810, 1957.

Another important contribution to the understanding of the mechanism for lipid transport in human blood is made in this publication. The unesterified fatty acid (UFA), often referred to as non-esterified fatty acid (NEFA) or free fatty acid, is taking on more and more importance in over-all fat metabolism. Although it constitutes a very small percentage of the normal serum lipid (less than 10 per cent) it would appear to be a highly dynamic material with a rapid turnover.

Dr. Gordon, following up previous work by himself as well as others, has shown in this paper a definite arteriovenous difference in the plasma UFA of blood in the lower extremity. Although these differences might appear small at first glance, they are probably quite significant in view of the rapid turnover rate. These A-V differences could be abolished by the administration of intravenous glucose and insulin. The above results suggest that tissue in the leg, presumably adipose tissue, is a source of extractable energy in the form of UFA, but is utilized only when necessary and that other readily utilizable energy will result in a decrease in UFA. This is further suggested by the observations that feeding either glucose or amino acids will result in a decreased plasma UFA as compared to the fasting state.

The role of UFA in over-all fat transport remains to be elucidated. Does UFA function only as a transport mechanism from adipose tissue in the starved state or is it an important mechanism in transport from the gastroenteric tract to liver and from liver to fat depots? Certainly the intravenous administration of large amounts of fat elevates the UFA only slightly, suggesting that it is of minor importance in the actual mechanism of clearing lipemia. However, again the problem of rapid turnover may interfere with the proper interpretation. Certainly more studies concerning the role of UFA in fat transport are indicated.

J. F. MUELLER

Early Effects of Fat Ingestion on Lipids and Lipoproteins of Serum in Man. Early Effects of Fasting and of Carbohydrate Ingestion on Lipids and Lipoproteins of Serum in Man. R. J. Hovel. *J. Clin. Investigation* 36: 848, 855, 1957.

These two companion articles deal with the problems of lipid transport. The first paper describes two experiments performed on healthy subjects. In the first experiment four subjects were maintained on average, low- and high-fat diets for varying periods and then fed a single high-fat meal containing 36 per cent cream. Total cholesterol, phospholipid and neutral fat in the blood were measured at 0, 4, 8, 12, and 24 hours. The low-fat diet (0.1 g/kg) resulted in a lowering of the cholesterol and phospholipid with a surprising elevation of neutral fat. The effect of the fat load, however, was comparable after each dietary period. The concentration of phospholipids rose uniformly as did the neutral fat, but the former stayed elevated much longer

than the latter. The cholesterol concentration rose only moderately in the two female subjects.

In the second half of the study, five subjects were given a single fat meal of varying composition. The various lipid components were measured in the very low density, low density and high density lipoprotein fractions at varying intervals after the fat meal. The major increment in total lipids was due to neutral fat in the very low density lipoproteins ($S_f > 10$). The cholesterol and phospholipids increased in this fraction also. The changes in the other fractions were much smaller, although there was some rise of the phospholipid in the high density fraction.

The fact that the lowest concentrations of neutral fat and very low density lipoproteins occurred three to four hours after a fat-free meal led to the study reported in the second paper. This had to do with the effects of fasting and glucose administration on the serum lipids. Seven normal subjects were studied. The total lipid concentration in very low density ($S_f > 10$) lipoproteins was variable during fasting, but fell consistently after 400 g of glucose had been ingested. All lipid constituents fell but the mean per cent fall was greatest in triglycerides, less in phospholipids and least in cholesterol. No change occurred in the S_f 0–10 fraction. The phospholipids showed a slight increase during fasting in the high density fraction and fell during glucose ingestion.

These short term experiments which were planned to study the role of various lipid components in fat transport must be differentiated from the long term effect of various dietary regimens. In such studies as these the attempt is made to assay the role of each lipid in the physiology of fat transport from the gut to the liver or fat depots and back again. It would appear that the low density lipoproteins play a major role, although how critical a role is not known. The changes measured may be passive rather than dynamically active. Only further studies will answer this. J. F. MUELLER

A Relation Between Non-Esterified Fatty Acids in Plasma and the Metabolism of Glucose. V. P. Dole. *J. Clin. Investigation* 35: 150, 1956.

Most of the fatty acids in plasma are bound to protein. This non-esterified fatty acid fraction was studied in 82 subjects with various clinical disorders. It was found that the plasma concentration increased with fasting and especially after epinephrine administration. The levels fell after a mixed meal, oral glucose, or the administration of insulin. Corn oil failed to produce a significant change even when given in amounts sufficient to produce gross lipemia. These responses parallel the effects of glucose, insulin, epinephrine, and fasting on ketosis.

A new, rapid, sensitive method for the determination of non-esterified fatty acids is also described.

S. O. WAIFE

The feeding of high-fat diets has long been known to reduce the rate of carbohydrate utilization manifested by a decreased glucose tolerance curve. It has now been demonstrated that the converse may be true: namely, the feeding of carbohydrate retards the utilization of fats. These are examples of adaptation of cellular enzyme activity to environmental conditions.

Oxidation of Palmitic Acid 1-C by Tissues of Carbohydrate and Fat Diet-Adapted Rats. J. Tepperman, H. M. Tepperman, and M. P. Schulman. *Am. J. Physiol.* 184: 80, 1956.

Groups of adult male rats were adapted to isocaloric high fat and high carbohydrate diets for 4 to 6 weeks. Liver slices and segments of diaphragm were incubated in a medium to which an emulsion containing palmitic acid-1-C had been added. Respiratory CO_2 was collected, precipitated as $BaCO_3$, and the specific activity estimated. Liver slices of fat diet-adapted rats oxidized more of the added palmitic acid than did those of carbohydrate-fed controls. Diaphragms of the former group also oxidized more added palmitic acid but abstracted less glucose from the medium than did those of the latter. AUTHORS

Utilization of Exogenous Fat and Turnover Rate of Endogenous Fat in the Normal and Hypophysectomized Rat. L. W. Matthews, S. Spector, J. Lemm, and P. Olynyk. *Am. J. Physiol.* 188: 308, 1957.

The utilization of C^{14}-labeled trilaurin and tripalmitin administered by both oral and intravenous routes and the turnover rate of total body fat was studied in normal and hypophysectomized rats. The hypophysectomized rats were found to utilize significantly more fat per millimole of CO_2 expired than normal rats. This increased utilization of exogenous fat varied from 14 to 97 per cent for the various fats and routes of administration and was found in both fasting and fed rats. The turnover rate of endogenous total body fat was found to be almost twice as rapid in the hypophysectomized rats with the half-life of total body fat 9.0 ± 1.33 days as compared to 14.9 ± 0.15 days in the normal rats. The fat content of the hypophysectomized rats was 11.12 g/100 g body weight compared to 8.29 g/100 g body weight in the control rats. When both of these factors are taken into consideration, the hypophysectomized rats are found to utilize 0.86 ± 0.13 g of endogenous fat/100 g body weight/day and the normal rats only 0.38 ± 0.04 g of endogenous fat/100 body weight/day. In the presence of this marked increase in total fat metabolism in the hypophysectomized rats, there is only a slight hyperlipemia, which may be accounted for by an increased serum cholesterol, a decreased fat content of the liver and an increased total body fat. AUTHORS

The level of utilization of fatty acid by the heart may be quite high depending upon substrate availability.

Metabolism of Carboxyl-Labeled Short Chain Fatty Acids by the Isolated Dog Heart. H. M. Cavert and J. A. Johnson. *Am. J. Physiol.* 184: 582, 1956.

A comparative study has been made of the metabolism of short chain carboxyl-labeled fatty acids to CO_2 and to glycogen in the isolated dog heart perfused with heparinized blood by the Langendorff technique. In confirmation of previous work by others, at high concentrations of administered acetate-1-C this compound contributed from 30 to 40 per cent of respiratory CO_2. Increasing the level of administered acetate correspondingly elevates the contribution of acetate carbon to CO_2. The portion of total CO_2 derived from 2-, 3-, 4-, and 8-carbon fatty acids appeared comparable (5 per cent) at substrate levels of 1–2 mM/liter. However, at 12–14 mM/liter the values were 20, 5, 3, and 3 per cent of total CO_2 derived from the C-labeled carboxyl carbon of acetate, propionate, *n*-butyrate, and *n*-octanoate, respectively. On the assumption of equal rates of combustion of all carbon atoms in each compound, the percentages were 41, 15, 13, and 25 per cent, respectively, of CO_2 derived from the administered labeled substrate. Labeled sodium formate was not rapidly converted to respiratory CO_2 in a single isolated heart experiment. Carboxyl carbon from none of the labeled fatty acids contributed detectably to carbon of cardiac glycogen. AUTHORS

The Effect of Different Dietary Fats on Liver Fat Deposition. D. A. Benton, A. E. Harper, and C. A. Elvehjem. *J. Biol. Chem.* 218: 693, 1956.

This is a continuation of earlier attempts to characterize the deposition of lipids in the livers of rats on 9 per cent casein diets, paying particular attention to the influence of different fats.

The basic diet used in this experiment contained 9 per cent casein, 20 per cent fat, 66.6 per cent sucrose and was supplemented with methionihe and choline. The experimental period was two weeks in duration.

Liver fat levels of rats fed 20 per cent corn oil and 20 per cent margarine were considerably lower than those fed equal amounts of butter fat or lard. Feeding 10 per cent corn oil and 10 per cent butter gave intermediate values. Although threonine reduced the level of liver fat in each case, the differences between effects of added fats persisted. Liver fat values for animals which received 18 per cent casein were very low and there was no difference between the liver fat levels of animals receiving the different fats at this level of protein intake.

Supplementation with glycine caused a decrease in the liver fat level of animals that received threonine and in those that did not receive it. However, the difference between liver fat content of those receiving corn oil and those receiving butter fat was reduced only when both threonine and glycine were added to the diet.

When the fatty acids of butter fat and of corn oil were separated, esterified with glycerol and fed as 20 per cent of the diet, liver fat levels of rats fed these preparations were the same as those of rats receiving the fats from which they were prepared.

Reducing the level of casein from 9 to 7 per cent increased the difference between liver fat content of animals receiving butter fat and those receiving corn oil. An even greater difference resulted when 7 per cent casein was fed and choline was omitted from the diet. Omission of choline from the diet also produced a marked increase in the level of liver fat of both groups.

The fatty acids of butter, which were isolated and fed as glycerides, caused liver fat to accumulate to the same extent as did butter fat. The solid fatty acid fraction of butter caused a much greater accumulation of liver fat than did the liquid fatty acid fraction, and stearic acid caused a much greater accumulation of liver fat than did oleic acid. The unsaponifiable material of butter was without effect. M. K. HORWITT

Influence of Dietary Factors Upon Human Serum Lipoprotein Concentrations. A. V. Nichols, V. Dobbin, and J. W. Gofman. *Geriatrics* 12: 7, 1957.

The authors present studies of the effect of various diets on the concentration of serum lipoproteins in human volunteers. Five healthy human volunteers on an isocaloric diet high in animal fat had a definite elevation of the S_f 0–12 and S_f 12 to 20 lipoproteins compared to the levels on a diet equally high in vegetable fat or low in fat supplemented by carbohydrate. There was no difference in the lipoprotein levels on the latter two diets. "The difference between animal and vegetable fat appears to be caused by an unfavorable effect of animal fat rather than by a favorable effect of vegetable fat." A group of subjects on a weight reduction regimen taking a low-fat, low-carbohydrate diet showed a fall in the level of both S_f 0–12 and S_f 20 to 400 lipoproteins. The highest level of S_f 20 to 400 lipoproteins in this study was found in a group of patients on a high-carbohydrate low-fat diet. This suggested that the rise seen in S_f 20 to 400 was due to increased carbohydrate intake rather than a low fat intake.

"A dissociation exists between the effects of various dietary measures on the S_f 0–20 lipoproteins and the S_f 20 to 400 lipoproteins. On certain types of diets both classes change in the same direction; on others, one class may change in the opposite direction from the other. Since both classes are cholesterol-bearers, the dissociation could be obscured if the only analytic measurement available were the serum cholesterol level." This is undoubtedly true but it still does not answer the question as to which is the most significant measurement.

The authors also discuss, but obviously cannot answer, the results of long-term versus short-time dietary effects on the control of serum lipoproteins. Long-term studies are certainly needed before sweeping conclusions are made as to the beneficial effect of a particular diet.
K. R. CRISPELL

Considerable evidence has been presented to support the concept that unsaturated fatty acids affect a lowering of serum cholesterol levels. This action has been observed in the absence of rigid restriction of other dietary fats. The mechanism remains unelucidated and the following work suggests that the degree of unsaturation of the "protective" fats is the only important consideration.

"Essential" Fatty acids, Degree of Unsaturation, and Effect of Corn (Maize) Oil on the Serum-Cholesterol Level in Man. A. Keys, J. T. Anderson, and F. Grande. *Lancet* 1: 66, 1957.

It has been recognized for some time that different dietary fats have differing effects on the serum-cholesterol level, but the reasons for this are not clear. One suggestion was that the relative degree of saturation of the fatty acids was important—the greater the saturation, the greater the rise in serum-cholesterol; another that the amount of the "essential" fatty acid linoleic acid made the difference. The published evidence is confusing and the experiment reported here was designed to test these theories under controlled conditions.

Twenty-six physically healthy and "metabolically normal" schizophrenic men were the subjects. They were fed a standard daily diet containing 100 g of protein, 40 g of mixed food fats and 300 mg. of cholesterol plus 100 g of the test oil (corn oil, sunflower-seed oil, sardine oil or butter fat). Calorie balance was maintained by individual adjustments in bread and jelly. The results and their statistical validation are presented to show that both sardine oil (which has the highest iodine number) and sunflower-seed oil (which has the highest linoleic acid content) tend to increase serum cholesterol levels more than corn oil. That is, they do not support the view that the serum cholesterol level is a simple inverse function of either the essential fatty acid content or the degree of unsaturation of the fatty acids. Corn oil appears to have some other specific feature by which it lowers serum cholesterol. F. E. HYTTEN

The participation of adipose tissue in the over-all metabolic activity of the body is now an accepted thesis. The depot fat stores maintain their own dynamic equilibrium geared to the needs of other tissues for their products in a manner similar to that of protein and glucose arising from the liver.

The Metabolic Activity of Adipose Tissue—A Review. B. Shapiro and E. Wertheimer. *Metabolism* 5: 79, 1956.

One of the functions of body fat is that of providing a storage depot of energy. Adipose tissue has been shown to be metabolically active with its fat content in a constantly changing composition with respect to the individual fat molecules. This is shown by the incorporation of C^{14}-labeled acetate or glucose in a random fashion within the fatty acid chain. The synthesis of fat within adipose tissue is inhibited in fasting animals but is markedly enhanced after administration of insulin. Enhanced fat accumulation in adipose tissue is regularly preceded by glycogen formation. It is hypothesized that the precursor of fat formation is glycogen, the deposition of which is enhanced by insulin. The autonomic nervous system and especially epinephrine are important in regulating fat mobilization and deposition. Epinephrine increases liver fat and decreases carcass fat. In the hypophysectomized animal there is a delayed fat mobilization, while after anterior pituitary extract or growth hormone, fat mobilization is rapid with a loss of carcass fat, increase in liver fat, and ketosis. It is indicated that the adrenocortical steroids play a "permissive" role for the fat-mobilizing pituitary fraction. More work on the enzyme systems operating in fat metabolism within adipose tissue is needed. Glucomutase, phosphorylase, fatty acid dehydrogenases, and a fatty acid-activating system have been described in this tissue.

C. R. SHUMAN

Lipogenesis by Adipose Tissue, Dietary Effects. J. P. Miller, J. A. D. Cooper, and S. Freeman. *Proc. Soc. Exper. Biol. & Med.* 95: 817, 1957.

The effect of dietary factors upon lipogenic activity of adipose tissue was investigated in the fat tissue of the epididymis of normal, fasted, and fasted and subsequently re-fed rats of the Sprague-Dawley strain. The minced tissue was incubated in normal dog serum together with C^{14} labeled acetate. After preparation for examination, the material was analyzed for nitrogen, glucose, and lipid content, and the rate of conversion of carbon-1-C^{14} acetate into CO_2 and long chain fatty acids was determined *in vitro*. Fasting increased the nitrogen and decreased the lipid concentration and the lipogenic activity. In normal animals the lipogenic activity increased during recovery feeding subsequent to a period of fasting. Parallel to this increased activity was an increase in the nitrogen and glucose and a decrease in the lipid content. The lipogenic activity of the adipose tissue was fairly constant if the depletion of the fat stores was followed by recovery feeding.

M. SILBERBERG

Serum Lipids and Lipoproteins in Old Age. E. A. Nikkila and T. Niemi. *Scandinav. J. Clin. & Lab. Investigation* 9: 109, 1957.

Serum lipid and electrophoretic lipoprotein analyses were made of fasting blood samples of 38 elderly subjects 65 to 91 years of age, as a follow-up investigation of such serum components in normal people. At the same time an effort was made to determine the extent to which the sex difference in the increment of atherosclerosis at old age parallels changes in serum lipid and lipoprotein levels. The 18 women had significantly higher lipid levels than did the 20 men. In the female subjects there was a rise in serum lipids up to the 80th year; the rise ceased in men at age 65. The proportion of lipid in the lipoprotein was the same in both sexes. Changes of lipid levels occurred in both alpha- and beta-lipoprotein fractions. No real explanation is offered of the results. Women above age 60 do have less coronary atheromatosis than men even if they do have higher serum lipid levels than men of the same age. There may be a correlation between serum lipids and aortic atheromatosis, with a protective "factor" existing in the female coronary arteries that prevents

the expression of such a relationship in coronary atherosclerosis. Other workers fed chicks cholesterol and then administered estrogens; coronary atheromatosis was inhibited, but not lesions of the aorta. On the other hand, is the fall in the serum cholesterol level, after the age of 60 in human males, significant? Is it merely a statistical expression of the better survival of hypocholesteremic, rather than hypercholesteremic individuals? Mortality rate biostatistics do not seem to account for the phenomenon as a statistical anomaly. There is clearly a crying need for fundamental research on the biochemistry of aging, whether it be with rotifer or chick, as well as man. E. COHEN

The unsaturated essential fatty acids have not been shown to be a required dietary factor for the human adult although the suggestion has been made (prematurely) that arteriosclerosis may represent the result of a deficiency of these factors. In human infants and in certain experimental animals, investigators have found evidence indicating the need for essential fatty acids in the diet.

Effects of Essential Fatty Acids in Young Swine. E. G. Hill, E. L. Warmanen, H. Hayes, and R. T. Holman. *Proc. Soc. Exper. Biol. & Med.* 95: 274, 1957.

Young pigs obtained by hysterectomy three to six days prior to expected parturition were kept in individual cages for four weeks and then raised on purified diets free of essential fatty acids. These animals showed a high mortality, and those animals that survived beyond the weanling age, occasionally developed skin lesions. More often medial calcification of the aorta was found at or before 98 days on the experimental diet. Thus essential fatty acids are needed by swine for maintaining the desired state of health and tissue structure. M. SILBERBERG

Influence of a Dietary Supplement of Erucic Acid and Other Fatty Acids on Fertility in the Rat. K. K. Carroll and R. L. Noble. *Canad. J. Biochem. & Physiol.* 35: 1093, 1957.

Male and female rats of the Sprague-Dawley and Wistar strains were fed a stock diet supplemented with 10 per cent erucic acid. This C22-monoethylenic fatty acid is a prominent constituent of rapeseed and causes marked accumulation of cholesterol in adrenal cortex and increased excretion of cholesterol in rats maintained on a sterol-free diet. Males showed reduced spermatogenesis leading to sterility after five months' treatment. The effect is more marked in immature than in adult rats. If the damage is not too great, recovery may take place. In females thus treated the estrus cycle but not the parturition is disturbed. The offspring, however, does not survive well because of atrophy of the mammary gland and disturbed lactation of the mothers. A similar effect was obtained in females fed a diet supplemented with 15 per cent oleic acid or a diet deficient in essential fatty acids. Erucic acid probably interferes with the metabolism of essential fatty acids. M. SILBERBERG

Effect of Phenylethylacetic Acid and Its Amide (Hyposterol) on the Circulating Lipids and Lipoproteins in Man. M. F. Oliver and G. S. Boyd. *Lancet* 2: 829, 1957.

It has been suggested that some aromatic acids, particularly phenylethylacetic acid and its amide, are able to correct the disturbances of blood lipids associated with coronary disease.

The subjects of this investigation were 24 hypercholesterolemic men with electrocardiographic evidence of an old myocardial infarct. They remained on their usual diets; six of them received 2.8 g of phenylethylacetic acid daily for four weeks and another six 5.0 g daily. A further six received 1.2 g daily of phenylethylacetic amide for 12 weeks and the other six 4.0 g daily for 16 weeks.

In four of the six receiving 5 g daily of the acid there was a fall of up to 12 per cent in plasma cholesterol and no change in lipoprotein ratio, but in five, symptoms of flatulence, nausea, and vomiting were sufficient to stop the trial. One of the six men on the higher dose of amide had a good response: plasma cholesterol fell by 17 per cent, cholesterol-phospholipid ratio by 20 per cent and β-lipoprotein cholesterol by 15 per cent, but three of the six men felt unusually tired and sleepy.

In general, it is concluded that only a minority of subjects have a satisfactory biochemical response, but most of these suffer symptoms which make treatment impracticable. F. E. HYTTEN

Prefeeding of High-Fat Diet and Resistance of Rats to Intense Cold. J. LeBlanc. *Canad. J. Biochem. & Physiol.* 35: 25, 1957.

Albino rats weighing about 225 grams were fed a control diet containing 3.5 per cent fat and high-fat diets containing 17 per cent oil or lard, respectively, for 45 days. Subsequently, the animals were shaved, starved, and exposed to temperatures of 8° C until the rectal temperature had dropped to 32° C. In the controls the time required to drop 1° C was 0.34 hour; it was 0.82 in the animals kept on the high-fat ration. It is concluded that the latter animals used the fat reserves more readily than those kept on the low-fat diet. M. SILBERBERG

VITAMIN B_{12} ABSORPTION

Several methods have been devised for the measurement of the absorption of vitamin B_{12} using the radioactive cobalt labeled material. Among those most widely used at present are the urinary excretion technic of Schilling and the hepatic uptake determination, a new modification which is presented. The direct measurement of radioactivity in blood samples obtained following an oral test dose of the labeled vitamin has been employed successfully in several laboratories.

Differentiation of Macrocytic Anemias and Diagnosis of Pernicious Anemia and Sprue in Remission by Accelerated Measurement of Hepatic Uptake of Radioactive Co^{60} B_{12}. G. B. J. Glass and L. J. Boyd. *Ann. Int. Med.* 47: 247, 1957.

The author, who has been a pioneer in the use of hepatic uptake of radioactive Co^{60} labeled vitamin B_{12} in the diagnosis of various megaloblastic anemias, presents a modified version of the original technic. The purpose of the modification is to facilitate the test which has always taken longer than the fecal or urinary excretion tests. With the use of cathartics, the residual radioactivity in the bowel can be disposed of quickly and in 90 per cent of the patients an accurate assessment of the absorption of vitamin B_{12} can be made in 48 hours by most clinical isotope laboratories. Using this technic the author was able to differentiate pernicious anemia in relapse and remission, sprue and histamine-refractory anacidity as previously reported with other technics.

Thus another method is available for the ready diagnosis of pernicious anemia. Although the modification of the hepatic uptake technic does decrease the time previously necessary to do the test, we wonder if technically there are not more chances for error than with the urinary excretion test. Certainly as far as the patient's comfort is concerned it would not seem to be as easy to perform. It would appear that whichever test is decided upon each laboratory should adhere to it and learn to develop values for their own laboratory so that their results are always comparable.

J. F. MUELLER

Studies of B_{12} Co^{60} Absorption in Malabsorption Syndrome: Results Before and During Specific Therapy. J. W. Frost, M. I. Goldwein, and B. D. Kaufman. *Ann. Int. Med.* 47: 293, 1957.

Although the use of the vitamin B_{12}-Co^{60} urinary excretion test is well established for the diagnosis of pernicious anemia, its use in other malabsorption syndromes is yet to be proved. Previous reports have shown that in sprue, regional enteritis, and blind loop syndrome, the absorption of vitamin B_{12} was low and uncorrected by the addition of potent intrinsic factor. The authors report the use of this test in five patients with sprue, two patients with blind loop syndrome, four with regional enteritis, one with extensive small bowel resection, one with cytic fibrosis of the pancreas, and two each with subtotal gastric resection and gammaglobulinemia. There was a great deal of variability in the absorption of the labeled vitamin B_{12} both before and after therapy in all groups. In their opinion this test is unreliable in the diagnosis of malabsorption syndromes and in assessing the therapy utilized.

J. F. MUELLER

Vitamin B_{12} Absorption in Atrophic Gastritis. M. Siurala and W. Nyberg. *Acta med. scandinav.* 157: 435, 1957.

The absorption of Co^{60}-labeled vitamin B_{12} as shown by the urinary radioactivity test (U.R.T.) was studied in control subjects and patients with various types of "gastritis" revealed by gastric biopsy. Results were normal in patients with superficial gastritis but subnormal in atrophic gastritis. When atrophy was incomplete, normal values followed carbachol administration. With total gastric atrophy, which resembled the histologic picture of pernicious anemia, the U.R.T. was usually low and it responded to carbachol in only one patient. There was, however, an increase after intrinsic factor was given. These findings suggest that impaired vitamin B_{12} absorption occurs in atrophic gastritis and that it is due to decreased intrinsic factor production.

W. H. J. SUMMERSKILL

In pernicious anemia, impaired absorption of vitamin B_{12} is attributed to the absence of intrinsic factor associated with histamine-refractory achlorhydria. Increased absorption of the vitamin with oral histamine may be a nonspecific effect which should be tested with other substrates.

The Effect of Oral Histamine on the Absorption of Radioactive Vitamin B_{12} from the Gastro-intestinal Tract. K. B. Taylor and W. H. Taylor. *Clin. Sc.* 16: 401, 1957.

The effect of histamine on the absorption of $Cobalt^{60}$ labeled vitamin B_{12} was tested in 12 patients with pernicious anemia. In four of the patients appreciably more of the vitamin was absorbed when given with 3 mg of histamine phosphate; in the other eight there was no improvement in absorption even when 10 mg of histamine was given. Parenteral histamine gave no effect in the four positive cases but in two of them a repeated trial with oral histamine also failed to show enhanced absorption of vitamin B_{12}.

Possible mechanisms are discussed but no suggestion fits all the facts. Two main conclusions are drawn: first, that the effect is too inconstant to have any clinical value; and second, that since enhanced absorption may occur due to histamine, its presence should be tested for in preparations of intrinsic factor before they can be assayed.

F. E. HYTTEN

Enhancement of Vitamin B_{12} Absorption by Substances Other than Intrinsic Factor. S. M. Greenberg, J. F. Herndon, E. G. Rice, E. T. Parmelee, J. J. Gulesich, and E. J. Van Loon. *Nature* 180: 1401, 1957.

"Modified liptril," a D-sorbitol solution, enhanced very considerably the absorption of vitamin B_{12} in both stock rats and vitamin B_{12}-deficient rats. Certain other carbohydrates (D-mannitol, L-sorbose and D-xylose) tested subsequently had a similar action. As intrinsic factor is a mucoprotein, or a mucopolypeptide, with a high carbohydrate content, the carbohydrate portion may play the major part in effecting vitamin B_{12} absorption. D-sorbitol and other carbohydrates may protect vitamin B_{12} by forming a complex which

prevents more rigid binding by other substances, or they may stimulate absorption mechanisms in the gastrointestinal tract. This work, furthermore, raises prospects of practical oral therapy in the management of vitamin B_{12}-deficiency anemias.

W. H. J. SUMMERSKILL

Massive doses of vitamin B_{12} given orally to patients with pernicious anemia have been found to produce a rise in reticulocyte count and to be therapeutically effective despite the lack of intrinsic factor.

A Dual Mechanism of Vitamin B_{12} Plasma Absorption. A. Doscherholmen and P. S. Hagen. *J. Clin. Investigation* 36: 1551, 1957.

Eight patients with pernicious anemia and eight control subjects were carefully studied after the oral administration of test doses of vitamin B_{12} containing various quantities of radioactive-labeled cobalt. Essentially no plasma absorption was observed in patients with pernicious anemia when given small (0.56 μg) doses of vitamin B_{12} without intrinsic factor concentrate. However, when the concentrate was administered, absorption curves were found which were similar to those found in control subjects on the same dose. This curve showed a delayed plasma radioactivity peak.

Oral doses of 50 to 300 μg of vitamin B_{12} without intrinsic factor concentrate, when given to patients with pernicious anemia led to a different absorption curve. This curve was characterized by an early rise in plasma radioactivity and an early peak concentration. When intrinsic factor concentrate was given with vitamin B_{12}, the early rise in plasma level of activity was delayed and the hepatic uptake of radioactivity was enhanced. A slightly diphasic absorption curve of radioactive vitamin B_{12} in the plasma was found in control subjects. These findings support the concept that there are two mechanisms for the absorption of vitamin B_{12} in the gastrointestinal tract. S. O. WAIFE

The megaloblastic anemia of fish tapeworm infestation is indistinguishable from pernicious anemia, responding to treatment with vitamin B_{12} or folic acid.

The *In Vitro* Uptake of Vitamin B_{12} by Diphyllobothrium Latum and Its Blockage by Intrinsic Factor. G. Brante and T. Ernberg. *Scandinav. J. Clin. & Lab. Investigation* 9: 313, 1957.

It is well known that the fish tapeworm, *Diphyllobothrium latum*, in some way produces an anemia and a clinical syndrome essentially indistinguishable from pernicious anemia. In this study, it was found that expelled worms take up vitamin B_{12} from a fluid medium very efficiently. Intrinsic factor could block this uptake. These findings support the suggestion that *in vivo* the worm may compete with intrinsic factor for orally ingested vitamin B_{12} and in that way may cause the pernicious tapeworm anemia. S. O. WAIFE

Elevated serum levels of vitamin B_{12} have been reported repeatedly in patients with Laennec's cirrhosis. Despite this finding, parenteral administration of this vitamin has been used widely in treatment of hepatic disorders without convincing evidence of benefit.

Serum-Cyanocobalamin Level in Chronic Intestinal Disorders. M. J. Meynell, W. T. Cooke, E. V. Cox, and R. Gaddie. *Lancet* 1: 901, 1957.

A method of estimating serum cyanocobalamin level using the *lactobacillus leichmannii* and measuring growth of the culture by means of pH change is described. In 43 healthy persons, mostly medical students, and in a group of hospital patients used as controls, almost every level fell between 100 and 450 $\mu\mu$g/ml and this was accepted as the normal range. Levels below 100 $\mu\mu$g/ml were found in 36 of 78 subjects with idiopathic steatorrhea and in 26 of 43 with regional ileitis. In 13 of 20 patients with cirrhosis of the liver, levels exceeded 450 $\mu\mu$g. The results are discussed with particular reference to the results of other workers using different methods of assay. F. E. HYTTEN

Vitamin B_{12} Levels in Serum and Urine Following Parenteral Administration of Crystalline Vitamin B_{12} or Liver Extract. L. L. Sheely, O. N. Miller, and W. G. Unglaub. *Proc. Soc. Exper. Biol. & Med.* 94: 629, 1957.

Eight normal subjects were given 50 μg of crystalline vitamin B_{12} and a similar group was given an amount of purified liver extract containing 50 μg of this vitamin. The total and bound serum vitamin B_{12} levels and the urinary excretion of vitamin B_{12} were compared in the two groups. The concentration of the free vitamin was highest in the group receiving crystalline vitamin. Over the 24-hour period, the percentage of bound vitamin in the serum was greater in the group receiving liver extract. Urinary excretion of vitamin B_{12} was significantly lower in the group receiving liver extract. These differences are probably due to slower absorption from the site of injection of the liver extract.

L. KINSELL

Megaloblastic anemia may develop three years or more after gastroectomy. The delay in onset may be related to the stores of anti-anemia principle found in normal liver or in the decreased demand for coenzymes derived from the vitamin in the patient whose dietary intake of protein may be deficient.

Incidence of Megaloblastic Anemia after Subtotal Gastrectomy. L. D. MacLean. *New England J. Med.* 259: 262, 1957.

Megaloblastic anemia indistinguishable from pernicious anemia eventually follows total gastrectomy or proximal subtotal gastrectomy with esophagoantrostomy. In this paper the incidence of megaloblastic anemia was determined in 1,500 patients who had undergone various types of distal subtotal gastrectomies and the intrinsic factor production was assayed by

measuring the absorption of radioactive vitamin B_{12} in 13 patients. Megaloblastic anemia developed in nine out of the 1,500 patients screened and only one of the 13 patients showed impaired absorption of radioactive vitamin B_{12}. Microscopic examination of the previously resected stomachs of these patients revealed an atrophy typical of pernicious anemia.

These results would indicate that pernicious anemia will develop in a few patients following subtotal gastrectomy which might not have developed otherwise and that it is possible to predict megaloblastic anemia after microscopic examination of the resected portion of the stomach. M. W. BATES

Histopathology of Hydrocephalus Resulting from a Deficiency of Vitamin B_{12}. P. M. Newberne and B. L. O'Dell. *Proc. Soc. Exper. Biol. & Med.* 97: 62, 1958.

Weanling female rats of the Wistar strain were fed a vitamin B_{12} deficient diet composed mainly of glucose and soybean meal, until they had reached a weight of about 170 grams. Control animals received the same diet supplemented with 30 μg of vitamin B_{12}/kg. About 10 per cent of the offspring of the vitamin-deficient animals were hydrocephalic at birth. In severe cases the cerebrospinal pressure was 350 mm H_2O. The enlargement of the ventricles varied from 1 to 2 mm in mild cases to 6 to 8 mm in severe cases with occlusion of the aqueduct. In control animals hydrocephalus was not seen. Microscopically, the cortical tissues were thinned out or disrupted, the ependymal cells flattened, degenerated or missing, while preserved ependymal and plexus cells had undergone fatty change. A mild glial reaction about the ventricles was observed. M. SILBERBERG

The Vitamin B_{12}-Binding Principle of Human Gastric Juice: Influence of pH on the Bacterial Absorption of Free and Bound B_{12}. R. Grasbeck. *Scandinav. J. Clin. & Lab. Investigation* 9: 50, 1957.

Some bacteria (*Lactobacillus* and *Escherichia* spp.) have been found to absorb free, but not bound vitamin B_{12}. The authors found that "bound vitamin B_{12}," unavailable to resting *L. leichmannii* cells at pH 5–7, was absorbed by the bacteria at a lower pH. The phenomenon, however, could not be reproduced with *E. coli* suspensions. Experiments using radioactive vitamin B_{12} suggested that the bacterial suspension had detached the bound vitamin and had not adsorbed the whole complexes.

This is a significant study of a successful vitamin B_{12} assay by microbial adsorption inhibition methods, or by dialysis methods. The "detachment phenomenon at low pH" may be a basis for the observation that Castle's intrinsic factor lacks hematopoietic activity at low pH. E. COHEN

Vitamin B_{12} Potency of Cheddar, Swiss, and Cottage Cheese. A. M. Hartman and L. P. Dryden. *Food Res.* 21: 540, 1956.

Vitamin B_{12} was determined in cheese samples by both microbiologic and rat assay methods. The investigators had some difficulty in reconciling the different results obtained by the two methods. Natural cheddar and cottage cheese were similar in potency—33 to 38 μg vitamin B_{12} per kg. Swiss cheese was higher—58 μg per kg. There was no increase in vitamin B_{12} when cheddar cheese was cured. Processed cheeses, cheddar and Swiss, were lower in vitamin B_{12} than the corresponding natural cheeses. The higher content of Swiss cheese was thought to be due to a synthesis of vitamin B_{12} by propionic acid bacteria involved in the manufacture of this kind of cheese. F. E. RICE

RECENT STUDIES ON ASCORBIC ACID

The administration of ascorbic acid has been demonstrated to elevate serum cholesterol. Whether this effect is one of enhanced cholesterogenesis or the liberation of preformed sterol remains to be determined. In the formation of cholesterol from acetyl groups ascorbic acid may function as a factor in the hydrogen-transfer system within the cells.

Cholesterol-Ascorbic Acid Relationship; Changes in Plasma and Cell Ascorbic Acid and Plasma Cholesterol Following Administration of Ascorbic Acid and Cholesterol. W. Booker, F. DaCosta, W. Jones, C. Froix, and E. Robinson. *Am. J. Physiol.* 189: 75, 1957.

Cholesterol when administered chronically in large amounts depressed the blood ascorbic acid content of cells or plasma, or both, in rabbits and guinea pigs. Ascorbic acid administered simultaneously with cholesterol seemed to reduce the effect of the cholesterol. Acutely, ascorbic acid administration caused hypercholesterolemia in rats (noted also in other laboratory animals and in humans). It is suggested that hypercholesterolemia following ascorbic acid administration may result from liver release of cholesterol, which may be related to adrenal hormone action. AUTHORS

In the scorbutic state, capillary defects, impaired wound healing, bruisability, and other deficiencies in the function and formation of the supportive tissues of the body involve the inability of cells to produce specialized protein and collagen. Among the possible causes of these alterations is the abnormal amino acid distribution observed in vitamin C deficiency.

Carbohydrate Metabolism and Phosphate Turnover Rate in Scorbutic Guinea Pigs. S. Lahiri and S. Banerjee. *Proc. Soc. Exper. Biol. & Med.* 93: 557, 1956.

Young, female guinea pigs were fed a scorbutic diet with or without the supplement of ascorbic acid for 25 days. In the scorbutic animals, the liver weights were increased, the inorganic content of phosphorus of blood and liver and hepatic glucose-6-phosphatase activity were increased, while the glucose-6-

phosphate, fructose-6-phosphate and fructose-1,6-diphosphate contents of the liver were decreased.

M. SILBERBERG

Studies on the Amino Acids in the Blood Plasma of Scorbutic Guinea Pigs. P. V. Rangneker and L. P. Duga. *Canad. J. Biochem. & Physiol.* 36: 25, 1958.

Scurvy was produced in male guinea pigs weighing about 400 g by feeding a diet of rabbit chow and water ad libitum. Controls were given the same ration, but also received daily intraperitoneal injections of 10 mg of sodium ascorbate. The animals were observed for 47 days during which time the scorbutic animals lost about 145 g weight. In the plasma of the scorbutic and non-scorbutic animals amino acid concentrations were determined. The scorbutic animals had no arginine, hydroxyproline, threonine, or cystine. The values for valine, leucine, alanine, glutamic acid, serine and glycine were lower and those for phenylamine, lysine and histadine higher in scorbutic guinea pigs as compared to those obtained in the non-scorbutic controls. These findings are of interest in view of the deficient collagen formation seen in scorbutic animals.

M. SILBERBERG

The Influence of Ascorbic Acid Upon the Liver. G. C. Willis. *Canad. M.A.J.* 76: 1044, 1957.

The effects of ascorbic acid deficiency on the liver were studied in guinea pigs. A fatty degeneration was found confirming reports of earlier investigators. Massive necrosis, acute non-fatty hepato-cellular degeneration and changes in hepatic reticulin were also found. The addition of choline or cystine to the diet in no way prevented or ameliorated the liver lesions caused by the ascorbic acid deficiency. M. W. BATES

Ascorbic acid functions as a coenzyme in the oxidation of L-tyrosine and phenylalanine. Incomplete oxidation of the phenolic amino acids is observed in vitamin C deficiency.

Some Investigations on the Metabolism of Phenylalanine and Tyrosine in Children with Vitamin C Deficiency. T. H. J. Husman and J. H. P. Jonxis. *Arch. Dis. Childhood* 32: 77, 1957.

Three scorbutic children (ages 7, 8, and 17 months) and one normal child (age 14 months) were given DL-phenylalanine and L-tyrosine by mouth and studies were made of plasma levels and urinary excretion of these and other amino-acids. The administration of 10 g DL-phenylalanine produced approximately equal rises in the excretion of α-amino nitrogen in scorbutic patients and in control. The urinary excretion of free amino-acids was strikingly altered, particularly following L-tyrosine load. The plasma concentration of other amino acids was not significantly altered by the administration of phenylalanine or tyrosine. The levels of these two substances were high during administration but less so in a scorbutic child than in the control. Phenylalanine increased the plasma level of tyrosine both in scurvy and control, whereas administration of tyrosine hardly affected the plasma level of phenylalanine in either. During tyrosine loading, urinary excretion of this substance was much greater in scurvy than in the controls, and this is attributed to decreased renal tubular reabsorption of tyrosine. When tyrosine was given, the excretion of tyrosyl substances rose about ten times as much in the scorbutic patient as in the control; following phenylalanine, the excretion was only slightly greater in the scorbutic. T. C. PANOS

There is no evidence that ascorbic acid is involved directly in the formation of oxysteroids nor that adrenal function is impaired in scurvy.

Adrenal Cortical Activity in Scorbutic Monkeys and Guinea Pigs. S. Banerjee and H. D. Singh. *Am. J. Physiol.* 190: 265, 1957.

Twenty-four-hour urinary excretions of 17-ketosteroids were determined in female guinea pigs and rhesus monkeys during the progress of scurvy. Urinary excretions of both corticosteroids and 17-ketosteroids were also determined in female guinea pigs during the progress of scurvy and in pair-fed normal controls. 17-ketosteroid excretion diminished in both guinea pigs and monkeys during the early stages of their depletion of ascorbic acid. The excretion of this steroid, however, increased tremendously in all the three monkeys and in seven of ten guinea pigs, just before the animals died of severe scurvy. In three guinea pigs, the excretion was considerably diminished when they became acutely scorbutic. The excretion of both corticosteroids and 17-ketosteroids increased to a considerable extent in four of five guinea pigs when they became severely scorbutic but in the remaining guinea pig both these excretions diminished when the animal became severely scorbutic. Inanition had little effect on the urinary excretions of these steroids. It is concluded that the levels of steroids of cortical origin in urine may not have any relation to the vitamin C nutrition of the body. Different degrees of stress produced in these animals due to vitamin C deficiency might be responsible for the variation in the excretions of these steroids. AUTHORS

The Vitamin C Content of the Cerebrospinal Fluid and Blood of Dogs in Relation to Intravenous Injection of Hypertonic Solutions. D. Giarnieri and P. L. Ipata. *Acta Vitaminol.* 1: 33, 1957.

After introduction by intravenous injection of hypertonic solutions of glucose, urea, and sodium chloride having the same osmotic pressure, there is a statistically significant increase of ascorbic acid in the cerebrospinal fluid. Glucose and urea increased the vitamin content

by 45 and 38 per cent respectively, while sodium chloride solution had a minor effect (17 per cent).

AUTHORS

Ascorbic Acid in Some Oleiferous Brassicas Cultivated in Pakistan. S. A. Tremazi. *Brit. J. Nutrition* 11:1, 1957.

Certain forms of oleiferous *brassicas* (brown-seeded sarson, Japan rapes and toria) are grown in Pakistan as oilseeds and fodder crops, and some varieties of these have been introduced recently as new crops in consequence of work done at the Agricultural Research Institute, Lyallpur. In the young stage the plants are eaten as vegetables; the nonfibrous stem known as *gandal* mixed with the young branches and leaves is cooked as *sag* by the villagers in winter. It is well known that leafy vegetables, particularly if eaten raw, form a valuable source of ascorbic acid in the diet, so the ascorbic acid content of the oleiferous *brassicas* mentioned above has been studied. The results of the work over a period of two years (1950–51) are summarized below:

The edible portion proved to have an outstandingly high content of ascorbic acid which did not diminish appreciably in the 24 hours after gathering, which is usually the maximum time before the vegetable is eaten. Loss on cooking was not great and was due to diffusion of ascorbic acid into the cooking water. The concentration of ascorbic acid was low in the mature seeds and increased greatly on germination. B. SURE

DENTAL MEDICINE AND NUTRITION

Among dietary components, carbohydrate is the main factor involved in the production of dental caries. The cariogenic carbohydrate diets do not produce tooth decay in the germ-free animal indicating that the presence of micro-organisms is required to cause destruction of dental substance. Carbohydrate foods of low clearance rate from the oral cavity may provide a better medium for growth of bacteria with the resultant higher concentration of lactic acid and lower pH.

Acid Production from Different Carbohydrate Foods in Plaque and Saliva. T. G. Ludwig and B. G. Bibby. *J. Dent. Res.* 36: 56, 1957.

The influence of various carbohydrate-containing foods on the pH of the dental plaque was studied. Subjects were selected for these trials on the basis of a preliminary study of the responsiveness of the pH of their dental plaque scrapings to environmental changes. All pH determinations were made after suspension of the scrapings in water in a one-drop glass electrode. All scrapings were made before the subject had breakfast. After a preliminary baseline pH reading, the subjects rinsed their mouths for two minutes with a 20 per cent glucose solution. At 5 minute intervals for one-half hour, a new dental plaque sample was collected from the same area of the mouth for another pH determination. The five subjects whose dental plaque pH readings were lowest after the glucose rinse were selected for the experimental trials.

On successive mornings, each subject had a sample of plaque material collected for the baseline pH determination. Then a liberal portion of one test food was consumed. During the following half-hour, pH measurements were made under the same conditions as after the glucose rinse. In the course of the series of comparisons, each subject was tested on different days with a fig sandwich cookie, toffee, bread and jam, ice cream, fried potatoes, apple, a cola beverage, grape soda and unsweetened canned orange juice. After consumption of most test foods, striking pH decreases in the plaque were observed, although at a slower rate than for the glucose rinse. Large variations occurred from one test substance to another. Fig sandwich cookie, toffee and bread and jam resulted in the largest decreases in pH. The solid foods, French fried potatoes, apple and ice cream gave rise to relatively little pH decrease. The influence of the fluids, cola beverage, grape soda and unsweetened canned orange juice was also low.

Lactic acid determinations were also made on the saliva at the same intervals after food consumption. A general relationship was found between the pH of the plaque and the amount of lactic acid in the saliva.

J. H. SHAW

Further Observations upon the Caries-producing Potentialities of Various Foodstuffs. T. G. Ludwig and B. G. Bibby. *J. Dent. Res.* 36: 61, 1957.

In previous studies from this laboratory, it had been postulated that the "caries-producing potential" of a food could be determined by multiplying the quantity of the food retained upon the teeth by the amount of acid produced on fermentation of the food in saliva. The present series of experiments was conducted to determine whether the reliability of laboratory determinations of the "caries-producing potentials" could be increased and also to evaluate the influence of several variables upon the determinations of these potentials. In the current studies, the acid production from the foods was determined at one time in aliquots of saliva taken from a single saliva pool to eliminate variations in saliva composition. In order to determine acid production from each food, a 3-g portion of the test food was emulsified in 100 ml of distilled water and from this mixture two 5 ml samples were taken and placed in sterile test tubes. Five ml of pooled saliva was added to each sample. One of these was immediately titrated with 0.1 N sodium hydroxide and the other was incubated at 37° C for four hours, after which it was titrated in the same fashion. Under these circumstances the greatest quantity of acid was formed from milk chocolate. Successively smaller quantities were formed from such dissimilar foods as toffees, white bread, dates and ice cream. Acid production values for beverages were low and the amount of acid formed from any food substance did not seem to depend solely on the percentage of carbohydrate in that food.

Tests were made to determine the extent of variation in food retention in the mouths of test subjects when consumed at different times on different days during a three-week period. Estimations made at different times on the same day were usually of the same order. However, estimations of retention at weekly intervals were subject to great variations. For example the retention values for doughnuts observed over a three-week period in the same individual ranged from 450 to 600 mg; for carbonated beverages from 210 to 360 mg. The extent of the variation was reduced by increasing the amount of the test food at a given time. For any particular food some subjects gave retention values of a considerably higher order than did others. Individuals who gave high values for one food usually gave relatively high values for all foods. Conversely, the subjects who gave low values for one food gave relatively low values throughout. Milk chocolate, cookies and ice cream gave the highest values for most subjects, while the lowest values were given by white bread, bananas and apples, and beverages.

The degree of retention in various areas of the mouth was studied in detail with seven different foods. Some foods were retained approximately equally upon the teeth and on the mucous membrane, while for others a much higher proportion of the food adhered to the mucous membranes than to the teeth. In the former class, were shortbread, cookies, dates and milk chocolate and in the latter case were white bread, ice cream, apples and sweet beverages. Retention values for solid foods were by and large much higher than for liquid foods.

These very considerable variations in food retention between different subjects from time to time, and for the same subject for different foods in the different areas of the mouth, suggest the real problems inherent in testing the cariogenic property of any food by strictly laboratory and physical procedures, without well-controlled trials on the actual ability to produce carious lesions in the human patient. J. H. SHAW

The Effect of Diet on the Deposition of Glycoprotein in the Teeth and Its Relationship to Dental Caries in the Syrian Hamster. J. D. Buxbaum, H. Kohn, L. M. Proutt, and R. H. Oster. *J. Dent. Res.* 36: 173, 1957.

These investigators studied the relationship of the sugar content of experimental diets to the concentration of glycoprotein in the teeth. Three diets were used which were designated as sugar-rich, sugar-deficient and control. Actually two formulae are given for sugar-rich diets, one of which contains 63 per cent sucrose and the other 51 per cent sucrose plus an additional 12 per cent of carbohydrates from powdered whole milk and alfalfa meal. No statement is made in the paper about which sugar-rich diet was used. The sugar-deficient diet contained no sucrose but did contain 35.6 per cent carbohydrates from powdered whole milk and alfalfa meal. The control diet was laboratory chow which contained 46.5 per cent carbohydrates. A total of three generations of hamsters was to have been raised on each dietary regimen but this was possible with the control diet only. On the sugar-rich diet or diets a second generation was possible but there were no offspring on the sugar-deficient diet. Glycoprotein determinations were made for each animal after 100 days of age on a pooled sample that included the enamel and dentin of incisors and molars, as well as intact and carious material. The average caries score in the 15 hamsters fed the sugar-rich diet was about 10 times as great as the average score of the 14 hamsters fed the control diet and 18 times as great as the average score of the seven hamsters fed the sugar-deficient diet. Glycoprotein values for the hamsters fed the sugar-rich diet averaged 0.71 mg per g of tooth substance in comparison with an average of 0.28 for the control hamsters and 0.13 for those fed the sugar-deficient diet.

The interpretation of these results is open to question because of the pooling of carious and non-carious material. Instead of stressing a correlation between the sugar content of the diet and the gylcoprotein content of the teeth, it would be more important to point out the correlation between the amount of carious material in the sample and the glycoprotein content of the teeth. Unless the carious material can be eliminated from samples of this sort before chemical determinations are conducted, no sound conclusions can be drawn.

J. H. SHAW

A high level of dietary tryptophan may increase the incidence of caries formation by promoting the growth and activity of oral bacteria or by changing the pH of the saliva.

Effect of Varying Levels of DL-Tryptophan in Diet on Dental Caries in Rats. W. Buttner and J. C. Muhler. *Proc. Soc. Exper. Biol. & Med.* 95: 309, 1957.

Rats of the Sprague-Dawley strain were kept on a cariogenic diet composed of yellow corn grits 52.7 per cent; ground yellow corn 11.3 per cent; powdered whole milk 30 per cent, alfalfa 4 per cent, iodized sodium chloride one per cent and irradiated yeast 0.2 per cent with the addition of 0.05 μg/ml fluorine to drinking water. Three groups of the rats received this diet supplemented with DL-tryptophan at 0.6, 1.8 or 5.4 g/kg levels, while one group remained as a non-supplemented control group. Addition of DL-tryptophan increased the incidence of dental caries. As the level of dietary tryptophan increased, there was a rise in salivary tryptophan excretion. These results are in apparent disagreement with increased salivary tryptophan levels observed in humans immune to dental caries. M. SILBERBERG

The Formation of Lactic Acid in Dental Plaques. I. Caries-Active Individuals. B. W. Moore, W. J. Carter, J. K. Dunn, and L. S. Fosdick. *J. Dent. Res.* 35: 778, 1956. **II. Oral Conditions of Primitive Bushmen of the Western Kalahari Desert.** A. J. Clement, R. Plotkin, and L. S. Fosdick. *J. Dent. Res.* 35: 786, 1956. **III. Caries-Immune Individuals.** H. R. Englander, W. J. Carter, and L. S. Fosdick. *J. Dent. Res.* 35: 792, 1956.

These papers are reviewed together as the purposes and the methods were the same in all three and only the nature of the experimental subjects varied. The 101 subjects in the first paper were 18 to 20 years old, recruits from the Great Lakes Naval Training Center, who were judged to be average or above average in their caries rate. In the second study, there were 50 primitive Bushmen, 21 males and 29 females, varying in age from about eight years to rather old individuals. No definite carious lesions were found in the teeth of any of these subjects in the course of the oral examinations. The 51 subjects in the third paper were selected from the personnel at the Great Lakes Training Center and ranged in age from 17 to 23 years. On the basis of the clinical and radiographic dental examinations, they were considered to be completely caries-free.

In all subjects, samples of plaque were taken from noncarious areas of each subject, before and at 5 min intervals for 20 min after a 1 min rinse with 50 per cent sucrose solution. The pH of each sample and its lactic acid content were determined. In the caries-active subjects the average pH of the plaque dropped from 6.3 before the sucrose rinse to a low of 5.6 at 15 min after the rinse. The average percentage of lactate increased from 0.3 before the sucrose rinse to a peak of 1.7 at 15 min after the rinse. Among the Bushmen, the average pH reading of the plaque before the rinse was 6.9 with a low of 6.5 at 10 and 15 min after the rinse. The average lactate value before the sucrose rinse was 0.2 with a peak lactate value attained at 0.6 at 15 min after the rinse. The average pH of the plaque material from the 51 American caries-free subjects was 6.8 dropping to a low of 6.7 at 5 min after the rinse that was maintained until 15 min after the rinse, after which the original average of 6.8 was noted. The average lactate content before the rinse was 0.4 per cent increasing to 0.7 at 10 and 15 min after the rinse.

The similarity of the data for the primitive Bushmen and the American caries-immune subjects and the dissimilarity of these data from those for the American caries-active subjects are obvious. J. H. SHAW

Vitamin A deficiency results in a failure of normal development of the ameloblasts. In experimental animals, the abnormality in ameloblast growth results in a reduction in formation or organic matrix of enamel. However, enamel hypoplasia due to vitamin A deficiency has not been well-documented in humans.

The Relation of Nutrition to the Morphology and Size of Rat Molar Teeth. K. J. Paynter and R. M. Grainger. *J. Canad. Dent. A.* 22: 519, 1956.

These studies on the relation of nutrition to the morphology and size of rat molar teeth represent a landmark in dental investigations. Vitamin A deficiency was imposed on female rats prior to mating and continuing through pregnancy and lactation. The offspring were killed at 20 days of age and detailed measurements made of the maxillary right first molar. Vitamin A deficiency during pregnancy and lactation resulted in the development of molars in the offspring that were appreciably smaller than those of offspring born to normal controls. This decrease in molar size was proportional to the decreased body weight of the vitamin A-deficient animals. In another experimental group of female rats, supplements of fluoride at 12 p.p.m. during pregnancy and lactation resulted in as large reductions in tooth size among the offspring without commensurate reductions in body weight. A high phosphate-normal calcium diet during pregnancy and lactation also caused a comparable reduction in molar size without any reduction in body weight of the offspring. When the latter diet was begun 10 weeks before conception and maintained until weaning, a still greater reduction in molar size was produced with a body weight reduction among the offspring comparable to that in the vitamin A-deficient offspring. In addition, the depth of the mesial fissure of the first maxillary molar was less and the angulation of the sides of this fissure was greater than was observed in either of the other experimental or control groups. It would be interesting also to know if the density of the enamel and dentin of the smaller molars from the experimental animals differed from that of enamel and dentin from the control animals.

For decades, the size of a tooth and its topographic form were believed to be determined by genetic forces which could not be overruled by infectious diseases or nutritional deficiencies. These studies strongly suggest that certain nutritional variants during tooth development produce major changes both in the size and the characteristic shape of the teeth. It remains to be seen what influences these changes have upon the caries-susceptibility of these teeth. Possibly just such variations as these may be the explanation for lower caries-susceptibility of European children during World Wars I and II and for children in areas with optimal water fluoride levels and also for the lower caries-susceptibility of animals fed natural diets during tooth development.

J. H. SHAW

A Study of Tin in Enamel. F. Brudevold and L. T. Steadman. *J. Dent. Res.* 35: 749, 1956.

In view of the interest in stannous fluoride as a component of dentifrices, these investigators studied the distribution of tin in teeth of different ages and in different areas of the tooth. Among unerupted teeth the tin content of the enamel was constant from the surface enamel to the inner layers. The level from one pooled sample to another varied from 0.5 to 1.5 parts per million (p.p.m.) The erupted teeth that were studied for tin content were divided into teeth that had been filled and those that were still intact. The filled teeth were divided into three groups; those from people less than 20 years of age, those from individuals between 20 and 29 years of age, and those between 30 and 49 years of age. The intact teeth were all from individuals over 50 years of age. Among the filled teeth there was a great deal higher level of tin than among unerupted teeth. In addition, the surface layer of enamel had higher levels

of tin than did the deeper layers. For example the surface layer of the teeth from the 30- to 49-year-old group contained 80 p.p.m. of tin, whereas the inner layer contained 16 p.p.m. Among the intact teeth, the surface layer contained 7.0 p.p.m., the second layer 2.0, and the third and fourth layers no detectable amount. Among mottled teeth, the surface layer contained 3.5 p.p.m. of tin, decreasing to 1.0 p.p.m. in the innermost layer. Probably the most interesting findings in this paper are the major increases of tin in the teeth that have been filled. Presumably these are due to a diffusion of tin from the amalgam filling material into the surrounding enamel. Since secondary caries frequently occur near the edges of amalgam fillings, this increased tin content in these areas would indicate that tin had no anticaries properties. J. H. SHAW

RUSSIAN NUTRITIONAL LITERATURE

Influence of the Quantity and Quality of Fat in the Diet on the Assimilation of Calcium in Growing Animals. E. N. Vasilev. *Vopr. Pitan.* 15: 11, 1956.

The amount of fat in the diet influences the assimilation of calcium in growing animals. It was found that the optimal percentage of fat in food was 20 per cent. On such a diet the assimilation of calcium took place at the rate of 0.05 g to 1 g of fat. Diets containing 40 and 60 per cent of fat were the least favorable, and were found to diminish the accumulation of calcium in the bones. Saturated fatty acids in the diet had no effect on the assimilation of calcium in growing animals.

ABST. SOVIET M.

The Dependence of Urinary Excretion of Vitamin B_2 on the Amount of Ascorbic Acid in the Diet. Y. F. Udalov. *Vopr. Pitan.* 15: 23, 1956.

The metabolism of riboflavin in man was found to be influenced by the amount of vitamin C in the diet. Increasing the amount of vitamin C in the daily food ration caused a rise in the urinary excretion of vitamin B_2. This implies a mutual interdependence of the metabolism of the two vitamins in man.

ABST. SOVIET M.

Physiological Effects of Low Caloric Diet Supplying Sufficient Vitamins and Minerals and an Amount of Proteins Near the Lower Limits of Accepted Requirements. A. I. Makarychev, V. S. Tongur, A. M. Stepanyan-Tarakanova, T. A. Braksh, and A. V. Chudinovskikh. *Vopr. Pitan.* 15: 18, 1956.

Four types of low-caloric diet were arranged. The total weight of each type of the daily ration was 250–260 g, and its volume not more than 350 ml; each contained 1,000–1,060 cal, a sufficient amount of vitamins, an average of 50 g of absorbable proteins, 45–50 g of absorbable fats, and 95–100 g of absorbable carbohydrates. The aim of the investigation was the study of the function of various organs during a short period (10 days) of calorically insufficient diet. Observations concerning psychologic and physical working capacity and subjective sensation of hunger were made. The persons taking part in the experiment felt quite well all the time; working capacity and memory remained normal; they slept normally. Some of them noticed a short period of muscle weakness. Sensation of hunger was present only during first few days of the experiment. Toward the end of the experimental period (10 days) when they were given an opportunity to partake of food in unlimited quantities, their appetite was poor and quickly satisfied. If the diet (10-day period) supplies insufficient calories, proteins near the lower limits of daily needs, and normal intake of vitamins and minerals, and if at the same time the usual work (in industry) is undertaken, some hunger is complained of and at times muscular weakness is observed. Toward the end of the 10-day period anorexia appears. The functions of internal organs, working capacity, general and work activity, and general mood of the individual undergo no change. After the experiment the body weight quickly regains its original figure. It follows that a diet lacking in calories but supplying all necessary vitamins and minerals and minimum requirement of proteins can be used for 10 days without any ill effects. ABST. SOVIET M.

The Cobalt Contents of Some Foodstuffs of Vegetable Origin. K. V. Meleshko. *Vopr. Pitan.* 15: 43, 1956.

Watermelons, pumpkins, red cabbage, spinach, and marrows contain very little cobalt. Large amounts of it are found in onions, radishes, beet roots, peas, oats, and strawberries. According to Peresadko (1953) cobalt reduces blood pressure in hypertensive disease. It seems that these data should stimulate research into the effects of ingestion of foodstuffs of high cobalt content, such as strawberries and oats, on the course of hypertensive disease. ABST. SOVIET MED.

ITEMS OF GENERAL INTEREST

Performance Capacity and Effects of Caloric Restriction With Hard Physical Work on Young Men. H. L. Taylor, E. R. Buskirk, J. Brozek, J. T. Anderson, and F. Grande. *J. Appl. Physiol.* 10: 421, 1957.

Work was assigned which required 1,200 cal/day. A pure carbohydrate diet and 4.5 g NaCl were furnished. On 580 cal/day there was no ketosis or liver damage. However, blood sugar levels decreased. Aerobic and anaerobic work capacities were maintained: this was not true of pulmonary ventilation oxygen debt and pulse rate. On 1,010 cal/day blood sugar levels did not fall and other parameters were normal. Although maximal oxygen intake/kg was not changed there was a gradual decline in total maximal intake. Grip strength was not impaired. The authors conclude, on the basis of these and previous studies, that when weight loss is 10 to 16 per cent there is a fall in maximal

oxygen intake and grip strength. Sufficient calories, salt (and vitamins) which prevent ketosis, dehydration and hypoglycemia will support adequate work performance capacity if the energy output is moderate.

M. J. OPPENHEIMER

Influence of the Salt Intake on the Loss of Scalp Hair. E. Foldes. *Acta med. scandinav.* 159: 147, 1957.

A 63-year-old man with a moderately advanced type of common baldness was studied from the point of view that salt metabolism may have affected his loss of scalp hair. Hair loss was measured by a technic described in the paper. The observations indicate that in comparison to losses on a salt-poor diet, loss of scalp hair was considerably increased on a diet unrestricted in the content of table salt. These observations were carried out on numerous occasions over many weeks, and the results had a high degree of reproducibility. An increase in the amount of hair loss was manifest after two days or more on an unrestricted salt diet while the return to "normal" figures was noticeable during the first week of the salt-poor diet and complete in one or two weeks. The author notes that adherence to a salt-poor diet significantly reduced the loss of scalp hair.

These fascinating observations, although based on a series of one case, certainly deserve further study because of the tremendous interest in methods of reducing the rate of loss of hair in middle-aged and elderly males.

S. O. WAIFE

Coffee with Cream as a Cause of Dyspeptic Distress. P. Brummer and E. Sysimetsa. *Acta med. scandinav.* 159: 141, 1957.

It has been commonly observed that in some people dyspeptic distress follows drinking coffee and cream more often than drinking black coffee. Yet, Roth, Ivy and Atkins have demonstrated that the average gastric secretory response to coffee, cream, and sugar is only 59.7 per cent that of the response to pure coffee.

The authors studied 23 subjects (11 of whom were x-ray negative), all with dyspeptic distress. Fluoroscopy of each subject was made after ingestion of black coffee and also coffee with cream. Examinations of subjects with fasting stomachs were at two-day intervals. They were given barium sulfate first, then 100 ml coffee made from 5 g of dry ground roast coffee. When cream was used, a 10 g amount was added to the coffee. Criteria were gastric peristalsis, tone, and emptying determined by fluoroscope during the first one-half hour following ingestion. Data were collected on some subjects 2, 5, 10, 15, and 30 min after ingestion of coffee.

The results indicated that the controls showed no difference in motility when they ingested coffee or coffee with cream. However, those who complained of gastric distress after coffee with cream showed a decrease in peristalsis and also delayed gastric emptying, but no such effects after black coffee. The cause is still not known nor is it elucidated by these experiments. The authors speculate that hypomotility, plus a lowered threshold for gastric and duodenal pain may be a basic cause of dyspeptic distress.

E. COHEN

The Effects of Reduction in Food Intake on Growth and Differentiation of a Squamous-Called Carcinoma in Mice, with Special Reference to Experimental Chemotherapy. A. E. F. Pearson. *Brit. J. Cancer.* 11: 470, 1957.

Fragments of homologous squamous cell carcinoma and of sarcoma 37 of mice of strain RIII were transplanted subcutaneously into the right flank, the graft measured about 1 mm^2 at the time of transplantation and after 7 days had increased to about 1.8 mm^2. At this time one group of animals was underfed, while another group received the diet in *ad libitum* amounts. After 25 days when the underfed animals had lost 20 to 25 per cent weight, the transplants were removed, measured and studied microscopically. In 6 of 20 underfed mice, the grafts had undergone complete regression and the remaining one was much smaller than in the *ad libitum* fed controls. Microscopically, in the underfed mice only the peripheral parts of the transplants showed cancer cells and they were undergoing advanced keratinization, while in the *ad libitum* fed animals actively dividing tumor cells were seen throughout the grafts with only small foci of keratinization. Restriction of food intake inhibited growth of the transplanted sarcoma but no regression occurred and histologic differences could not be established between the animals of the two groups. If *ad libitum* food consumption was resumed growth was reactivated. Since benzoquinone-dioxine lowered the food intake of mice thus treated and likewise inhibited growth of the transplanted tumors, it is felt that some of the chemotherapeutic agents may exert a nonspecific effect.

M. SILBERBERG

Alterations in Thyroid Function Following Surgical Trauma. A. E. Schwartz and K. E. Roberts. *Surgery* 42: 814, 1957.

An increase in the urinary excretion of nitrogen by injured patients was described about 30 years ago by Cuthbertson. Since then widespread changes in the metabolism of protein, fat, carbohydrate, water, and certain minerals and vitamins have been observed following injury. Alterations in activity of the hypothalamic-pituitary-adrenal axis in this circumstance is well established, but the evidence regarding thyroid function is controversial.

Schwartz and Roberts measured protein-bound iodine, plasma calcium, serum inorganic phosphate and plasma magnesium concentrations in the first postoperative day of 21 patients (presumably euthyroid) undergoing major surgery.

The most striking and consistent changes were rises in the PBI concentrations (averaging about 1.5 μg/100 ml) in the first three hours after operation followed by

gradual falls in the next 21 hr to levels slightly below the preoperative control. Serum phosphate followed a similar course in the majority of patients, while plasma calcium usually fell in the first three hours and then remained low. Plasma magnesium was consistently slightly low three hours after operation, but came back to the control level within the first day.

The mechanism or significance of the reported biochemical changes were not investigated, but the authors point out that plasma magnesium concentrations decrease in dogs given triiodothyronine and then infused with phosphates. S. M. LEVENSON

Effect of Protamine on Alimentary Lipemia. A. J. Day, G. K. Wilkinson, H. R. Gilmore, and C. J. Schwartz. *Circulation* 16: 72, 1957.

Total serum esterified fatty acids, cholesterol, and phospholipids were measured in the fasting state and at intervals up to 180 min after a standard fat meal (100 ml of cream), in three groups of nine individuals each: healthy medical students averaging 25 years in age, atherosclerotic patients with history of old myocardial infarction, with a mean age of 58 years, and "non-atherosclerotic" patients averaging 51 years in age. 150 min after the test meal, saline, 100 mg protamine sulfate, or 5,000 units of heparin were given intravenously.

Heparin (given only to the student group) produced a significant reduction in the serum total fatty acids. Protamine sulfate was followed by a significant elevation in the total fatty acids in the student group, but had no significant effect on the total fatty acids of the two groups of older subjects. Protamine significantly elevated the serum cholesterol and increased the visible lipemia of the three groups studied, but did not affect the serum phospholipids.

The authors interpret their findings as evidence of a deficiency of heparin with advancing age.

W. H. ABELMANN

Metal Fume Fever from Inhaling Zinc Oxide. L. C. Rohrs. *A.M.A. Arch. Int. Med.* 100: 44, 1957.

When zinc is burned, melted, or heated to greater than 930° F the oxide is formed as a fine dispersion of dry particles 1μ in size. The inhalation of this causes a common, transient, benign form of metal fume fever. The initial symptoms are a sweetish taste, dryness and irritation of the throat. This is associated with sustained tightness, constriction in the chest, and a dry cough. Several hours following exposure, lassitude, malaise, headache, low back pain, and muscle cramps are noted. Chill and fever may appear which is accompanied by nausea and vomiting. On examination the patient has fever, perspiration, dyspnea, rales throughout the chest, and tachycardia. There is a polymorphonuclear leukocytosis. The x-ray of the chest is invariably normal or reveals only increased bronchovascular markings. Such an attack may last from 6 to 12 hr and rarely 24 hr. Weakness may follow but recovery is complete. A tolerance is developed to repeated exposure.

Three cases are reported. Two of the cases were caused by the dust from zinc-coated surfaces when cleaned with a steel wire buffing machine. One of the cases appeared to have rapid improvement when large quantities of milk were taken.

The most tenable theory of pathogenesis attributes the symptoms to the absorption of the proteins formed following the destructive action of zinc on the alveoli.

L. KINSELL

Effects of Gossypol in Young Chickens with the Production of a Ceroid-Like Pigment. R. H. Rigdon, G. Crass, T. M. Ferguson, and J. R. Couch. *A.M.A. Arch. Path.* 65: 288, 1958.

Gossypol, which is found in the gossypol glands of cotton seed, produces intoxication and a variety of disorders in many species such as hog, dog, cat, rabbit, guinea pig, rat, mouse, chicken, and fish. Typical manifestations of the disorder are loss of appetite, arrest of growth, nerve degeneration and paralysis, circulatory disturbances, ulcerations in the gastrointestinal tract, liver necrosis, and hemorrhages. The present investigation deals with the effect of gossypol on red blood cells and viscera of young Hyline white Leghorn and Red Chicks. The animals were fed a stock diet supplemented with gossypol in doses varying from 0.01 to 0.4 per cent for periods up to nine weeks. Depending on the dose and the duration of the experiment hemolytic anemia developed on as little as 0.03 per cent gossypol in the diet. In the duodenal villi and sinusoids of liver and spleen a yellowish-brown ceroid-like pigment is identified. These findings suggest that gossypol effects the erythrocytes to produce this pigment particularly since supplements of iron salts counteracted the production of anemia in the gossypol-fed chick.

M. SILBERBERG

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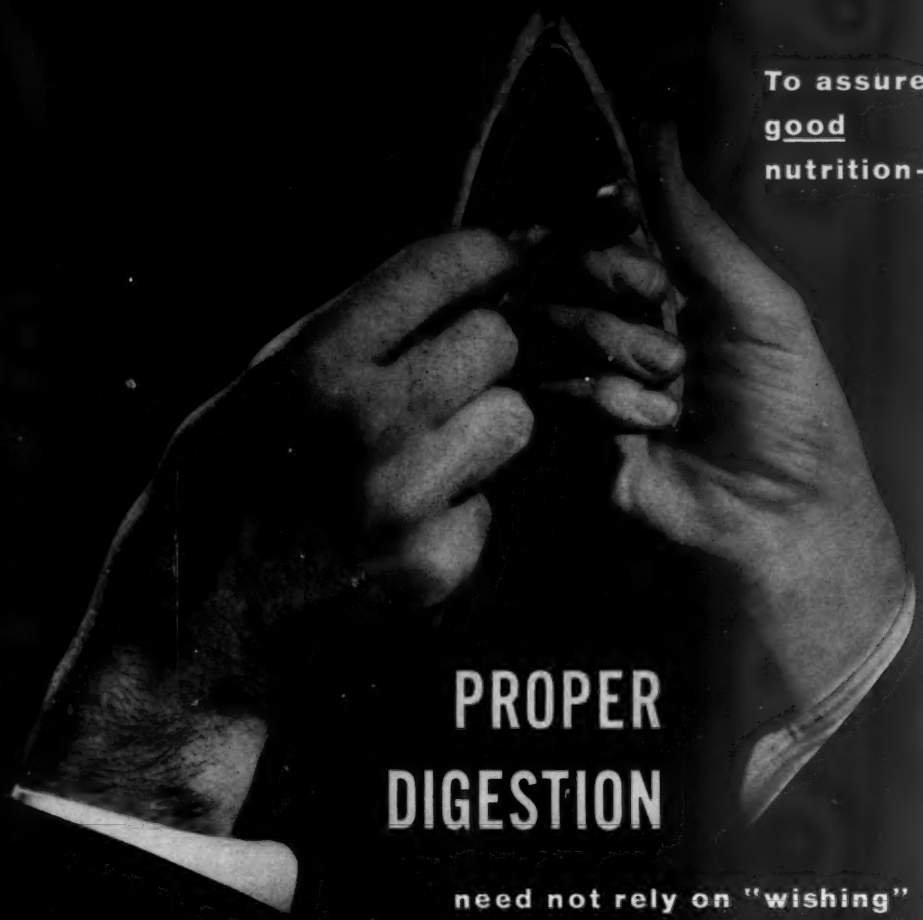

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Season's Greetings

American Journal of Surgery

American Journal of Medicine

American Journal of Cardiology

American Journal of Clinical Nutrition

Drugs and Modern Drug Encyclopedia

FOR PROMPT UTILIZATION

AND BETTER STORAGE

HOMAGENETS®

The only homogenized vitamins in solid form

Homagenets are unusually palatable—and good taste is especially important to your patients. Of more interest to the physician is the homogenization process. This presents both oil and water soluble vitamins in microscopic particles. Thus the vitamins in Homagenets are better absorbed and utilized—and stored longer.[1] These are definite advantages to your patient.

1. Lewis, J.M., et al.: J. Pediat. 31:496.

THE ADVANTAGES OF HOMAGENETS

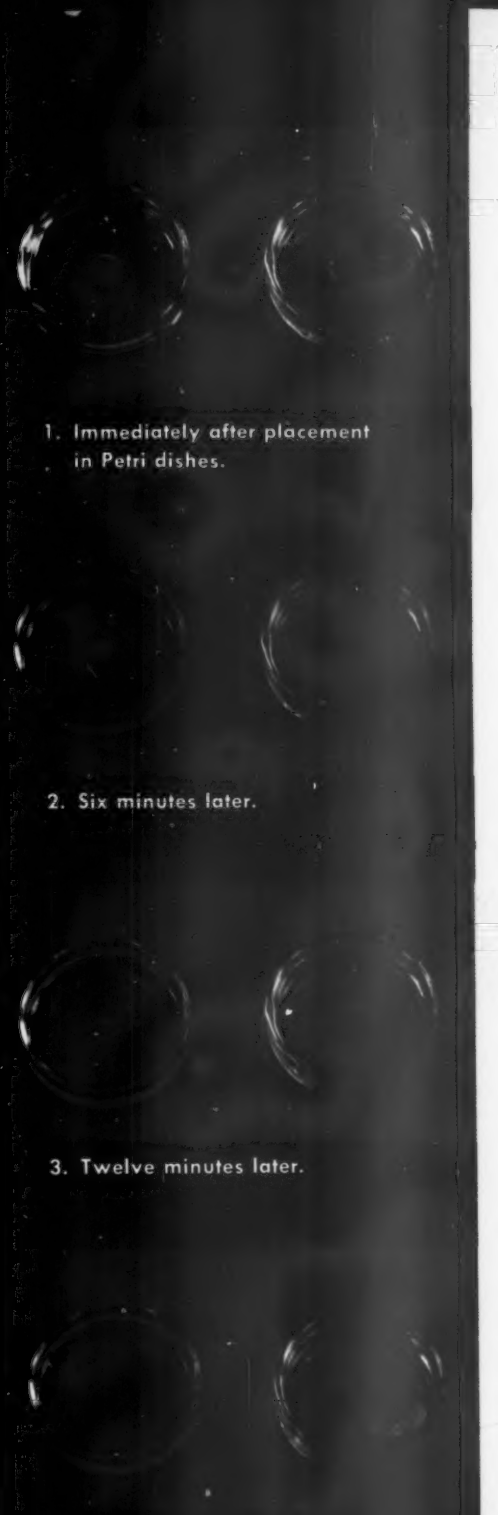
- Pleasant, candy-like flavor
- Better absorbed, better utilized
- Excess vitamin dosage unnecessary
- Longer storage in the body
- No regurgitation, no "fishy burp"
- May be chewed, swallowed or dissolved in the mouth.

Homagenets are available in five formulas: Prenatal, Pediatric, Therapeutic, Geriatric and Aoral (brand of vitamin A).

THE S. E. MASSENGILL COMPANY
Bristol, Tennessee

TURN THE PAGE
for laboratory proof of
the prompt dispersion
of Homagenets

VISUAL PROOF OF THE RAPID DISPERSION OF HOMAGENETS®



1. Immediately after placement in Petri dishes.
2. Six minutes later.
3. Twelve minutes later.
4. Two hours and 12 minutes later.

These photographs show the dispersion time of a Homagenet and a soft gelatin capsule in artificial gastric juice at 37°C.

Homagenets are available in five formulas: Prenatal, Pediatric, Therapeutic, Geriatric and Aoral (brand of vitamin A).

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BRISTOL, TENNESSEE

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nutritional therapy *in convalescence*

to accelerate convalescence...

Sustagen®

Complete food, Mead Johnson
powder

Builds tissue, promotes well-being, enhances rehabilitation

Specific treatment may vary infinitely in details, but one factor remains constant—the urgent need of every seriously ill patient for *therapeutic nutrition.*

With Sustagen you can give your patients extra nutritional reserves which they need to withstand both medical and surgical crises and debilitating diseases.

Sustagen in itself is a balanced diet and can be given by mouth or by tube. It can be used alone or as a supplement to the diet, for short term or prolonged nutritional therapy. Sustagen supplies every known essential nutrient for maintenance or rehabilitation.

Detailed information on the use of Sustagen in many clinical conditions is provided in the booklet "Nutritional Therapy: The Use of Food in the Management of Illness and Injury." Your Mead Johnson Representative will gladly supply you with a copy...or you may write to us, Evansville 21, Indiana.

Mead Johnson
Symbol of service in medicine

SUS15

The Vital Story

A Puzzling Medical Case. A sick, fretful baby lay on his back in a hospital crib recently, his legs extended and his knees slightly bent. He cried and tried to draw away from the examining physician. The doctor studied the case carefully, noting the symptoms and investigating the history of the little patient. Then he made his diagnosis. The baby had *scurvy*.

An Ancient Disease. To us scurvy is a remote disease. We know that years ago it attacked sailors and explorers living on monotonous diets of salt meat and hardtack. But it's hard to believe that scurvy occurs even today in the United States. To be sure, there are few cases, but there should be none! Scurvy is a disease resulting from a deficiency in the diet; it is simply the lack of enough vitamin C.

Scurvy was a disease of the Crusades. It traveled with Vasco de Gama around the Cape of Good Hope, killing two-thirds of his crew. It struck down a quarter of Cartier's men wintering in Canada in 1535. It occurred in famines and pestilences.

Vitamin C Isolated. Long before vitamin C was known, man found certain fruits and vegetables helpful in preventing and treating scurvy. These had been a remedy for scurvy for about 300 years when, in the first quarter of the Twentieth Century, researchers began to examine foods to discover the factor which made them antiscorbutic.

Between 1918 and 1925, Zilva concentrated this antiscorbutic substance from lemons and Agopian isolated it in cabbage.

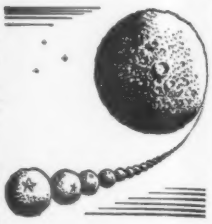
Zilva's work established the important properties of the vitamin, its molecular construction, and its activity as a "reducing factor" — its ability to absorb oxygen. In 1928 Szent-Györgyi, after eight years of investigation, isolated a strong reducing compound from adrenal glands, oranges and cabbage, which he called "hexuronic acid."

In 1931 C. G. King established the chemical structure of vitamin C, thus opening the way to new and important findings. The following year various investigators identified the "reducing factor," "hexuronic acid" and vitamin C as the same compound.

Nature's Vitamin Duplicated. Soon after the vitamin had been isolated and its chemical structure understood, it was synthesized by Reichstein and his fellow workers in Switzerland. Almost simultaneously in Great Britain, Haworth and his associates achieved the same results. The year was 1933.

The synthesis of vitamin C led to pilot plant and then to commercial production, making it available at lower costs than if it had been extracted from natural sources. In fact, the Reichstein synthesis is the basis for weekly production *by the tons* by the firm of Hoffmann-La Roche, the world's largest producer of vitamin C. This commercial production of vitamin C has had incalculable value to the health of people all over the world.

To The Moon And Back. In the United States today, the average *weekly* production of man-made vitamin C now approaches 40 tons — the amount contained in approximately three-quarters of a billion average oranges.



A graphic way to illustrate the huge annual production is to show the equivalent in oranges. Laid side by side, these oranges would reach over two million miles, or more than four round trips to the moon. The *weekly* production alone, expressed as oranges, would circle the world at the equator over one and one-half times.

Synthesis on such a gigantic scale has led to economical costs. Because of this and its proven value to health, vitamin C has an increasingly widespread use in food and pharmaceutical products. There is no substitute for Vitamin C!

A Little Goes A Long Way. One kilogram (2.2 pounds) of pure vitamin C will supply 13,333 people for one day with the amount recommended (75 milligrams) for daily intake by the National Research Council. Or it will supply 92 people for one year with the adult Minimum Daily Requirement (30 milligrams) set by the U. S. Food and Drug Administration.

Names. The designation, vitamin C, is used interchangeably with ascorbic acid. "Ascorbic" is a relatively new word in our language. It was devised by Szent-Györgyi and Haworth in 1933 from antiscorbutic.

Among chemists and nutritionists, vitamin C is known as *l*-ascorbic acid. The formula is $C_6H_8O_6$, which means that it is composed of carbon, hydrogen, and oxygen.

Physical Characteristics. *l*-Ascorbic acid is a white crystalline compound with a slightly acidic taste. It is readily soluble in water, alcohol, glycerin and propylene glycol, and insoluble in oils, fats, ether, benzene or chloroform.

Since it is able to "attach" itself to oxygen it is one of our most important antioxidants for certain foods that have a tendency to discolor and lose their natural flavor.

The designation "U. S. P." used with *l*-ascorbic acid indicates that the product conforms to the stringent specifications of the United States Pharmacopeia. These initials stand for top quality.

Vitamin C made by the Roche process is chemically and biologically identical with nature's own product.

How Vitamin C Helps Our Health. Vitamin C is a vital substance; we cannot live without it. Not only does it prevent or cure scurvy, but it is essential for maintaining healthy bone tissue, and for forming collagen, an important protein found in our skin, tendons, bones, cartilage and connective tissue. Vitamin C is needed for the healthy functioning of the vessels

Expanded Version

# of VITAMIN C *by* Science Writer

(*l*-Ascorbic Acid)

through which our body fluids flow. It also promotes healing of wounds and fractures. It is believed to be essential for tissue respiration, the continual action of the cells as they burn food materials to provide heat and energy for the body.

Our Daily Requirements. Even though ascorbic acid is found in all body tissues and fluids, it will be rapidly exhausted if we do not replenish our supplies each day.

The Food and Drug Administration of the U. S. Department of Health, Education and Welfare has established a *Minimum Daily Requirement* for adults of 30 milligrams. This is just enough to prevent recognizable signs of vitamin C deficiency.

The Food & Nutrition Board, National Research Council, recommends in its 1958 publication #589, these daily dietary allowances which are well above the F. & D. A. minimum:

Recommendations for Daily Vitamin C Intake
Ascorbic Acid (milligrams)

Men, all ages	75	Children, age 4-6	50
Women, all ages	70	Children, age 7-9	60
Women, pregnant (second half)	100	Children, age 10-12	75
Women, lactating	150	Boys, age 13-15	90
Infants, to one year	30	Boys, age 16-19	100
Children, age 1-3	35	Girls, age 13-15	80
		Girls, age 16-19	80

Use in Medicine. For some conditions, ascorbic acid is a necessity; for others, it is a help in restoring the body to normal. It is obvious that vitamin C is the cure for scurvy. Medical science has also found it to be helpful in the treatment of burns, in the repair of fractures, in high fever, in shock, in surgery and in exposure to extreme temperatures.

Of special importance is the use of ascorbic acid by pregnant and lactating patients. The physician must see that the infant receives his share of the essential vitamin, and that the mother's supply is not depleted.

Pharmaceutical manufacturers prepare many forms and potencies of vitamin C for the physician to prescribe.

Which Foods Are Best? Vitamin C is present in many foods. Citrus fruits — oranges, lemons, grapefruit, etc. — are generally good sources. Other fruit, certain green vegetables, tomatoes and potatoes are also sources of the vitamin. There are wide differences, however, between foods and even between varieties of the same food. The differences among commercially canned juices are graphically shown in the following table compiled by the U. S. Department of Agriculture:

Juice	Minimum	Maximum
	mg. ascorbic acid/100 mg. juice	
Apple	0.2	3.6
Grape	0	4.7
Grapefruit	10.0	49.0
Orange	9.7	70.0
Pineapple	5.4	18.0
Tomato	2.5	32.0

A Public Health Problem. It's an ironic situation that Americans, who have a plentiful and varied diet available to them, have been found by responsible researchers to have subclinical and recognizable vitamin C deficiencies.

The public relies on processed juices to supply a large part of its daily vitamin C requirement. All unknowing, it often fails to receive even a token amount because of the variations shown.

For these reasons — *widespread deficiencies and inadequate intake* — it is desirable to standardize the vitamin C content of processed fruit and vegetable juices at levels which will guarantee a significant amount of the vitamin per each serving of juice. Diet experts believe that such standardization is in the public interest. It may be achieved by the simple addition of pure, crystalline vitamin C. The Canadian government has recognized the value of this idea and has set a standard which all "vitaminized" apple juice must meet.

Saved: Natural Color and Flavor. Many delicious fruits which are quick frozen lose their natural color and delicate flavor during thawing. By adding pure *l*-ascorbic acid to these before freezing, processors can protect the natural color and flavor during thawing and for some time after. The ascorbic acid acts as an antioxidant; it "takes up" the oxygen in the air before that oxygen can trigger the reactions which cause the undesirable changes.

Vitamin C has been used for more than 15 years as the preferred antioxidant in frozen peaches, apricots, apples, berries, bananas, pears, pineapple, fruit purees and fruit cocktail. It has been used also in canned fruit and fruit juices, sauerkraut, pickles, beer, cured meats, milk, and mushrooms.

Homemakers seeking variety in the fruit and fruit juices served to their families will be influenced to buy the product to which sufficient *l*-ascorbic acid has been added to allow a label claim. When a nutritive claim is made for a food, a declaration must appear on the label. One form reads:

> *"One four-ounce portion provides 100% of the minimum daily adult requirement of vitamin C."*

Processors using the twin benefits of *l*-ascorbic acid — nutritive value and antioxidant effect — are delivering more attractive, more palatable, more popular, and more valuable foods.

Yours For the Asking. Summarizing our present knowledge of this vitamin, this article is one of a series published by the Vitamin Division of Hoffmann-La Roche Inc. Reprints of this, and all others in the series, are available without charge. Reasonable quantities will be available to those in education who wish to use the material in their teaching. Send your request to Vitamin Division, Hoffmann-La Roche Inc., Roche Park, Nutley 10, New Jersey; in Canada to Hoffmann-La Roche Ltd., 1956 Bourdon Street, St. Laurent, Montreal 9, P.Q.

"Just a Sandwich"

But Such Excellent Nutrition

So often, people say, "I'll have just a sandwich." A sandwich made with Enriched Bread has nutritional advantages over many a knife-and-fork meal.

In addition to providing an excellent vehicle for other nutritionally valuable foods, the nutrient values of Enriched Bread add a definite plus for good nutrition.

Enriched Bread

supplies growth-promoting protein, readily available energy, important B vitamins (thiamine, riboflavin, niacin, pantothenic acid, choline, folic acid, and other B-complex factors), iron, and calcium.

Enriched Bread is more than a compatible vehicle for other foods; it is an effective provider of essential basic nutrients.

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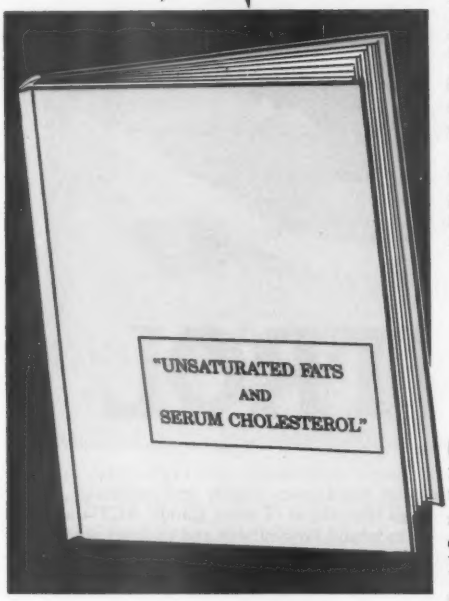

"Unsaturated Fats and Serum Cholesterol"

A review of the latest concepts and results of current research

This new book contains the most up-to-date bibliography of current research on: 1. The origin and behavior of cholesterol in the human body; 2. The effect of different dietary fats on serum cholesterol levels; 3. The nature of the active components in vegetable oils; and 4. Suggestions for practical diets.

Now ready for distribution to Physicians by the makers of MAZOLA Corn Oil, this book supplements the 1957 monograph, "Vegetable Oils in Nutrition" and provides a broader coverage of this important subject.

As a regular part of daily meals

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MAZOLA CORN OIL . . . the only leading oil made from golden corn, is rich in the important unsaturated fatty acids—When an adequate amount of Mazola is part of the daily meals, elevated serum cholesterol levels tend to be lowered . . . normal levels tend to stay level . . .

MAZOLA CORN OIL is a natural food, *and* cholesterol free, can easily be included as part of the every day meals . . . simply and without seriously disturbing the patient's usual eating habits . . . in salads, baking and other cooking processes.

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Technical Pamphlet, "Facts about MAZOLA Corn Oil," also available. Provides technical information on chemical and physical properties. Check here if you wish a copy of this pamphlet ☐

CORN PRODUCTS REFINING COMPANY

Each **TABLESPOONFUL** *of* **MAZOLA**

Provides approximately:

LINOLEIC ACID	**7.4 Gm.**
Sitosterols	130.0 mg.
Natural tocopherols	15.0 mg.
Cholesterol	0
Weight 14 Gm.	Calories 126

Total unsaturated Fatty Acids—85%

TYPICAL AMOUNTS PER DIET

For a 3600 calorie diet	3 Tbsp.
For a 3000 calorie diet	2.5 Tbsp.
For a 2000 calorie diet	1.5 Tbsp.

Hormones control blood and tissue levels of fats and related substances such as cholesterol . . . rate at which these are synthesized and destroyed . . . and manner in which they are utilized for body functioning. Fat metabolism is influenced by secretions of the pituitary, thyroid, adrenals, ovaries, testes and pancreas, and by action of liver and possibly kidneys.

Diseases of the endocrine glands may lead to serious disturbance of metabolism . . . of fats as well as carbohydrates and proteins. In diabetes, blood serum levels of cholesterol and other lipids are high . . . and can be changed by administration of insulin. In hypothyroidism, there is a tendency for fat to accumulate in body tissues and for serum cholesterol levels to be high. Administration of thyroxin stimulates an increase in metabolism . . . tends to lower serum cholesterol . . . and increases mobilization and oxidation of fat from body tissues.

The "master gland" . . . the pituitary . . . influences fat metabolism directly and indirectly . . . through stimulation of other glands. ACTH appears to inhibit fat synthesis and to lower serum cholesterol levels . . . probably by stimulating production of cortisone and other adrenal hormones. The "growth hormone" and "sex hormones" tend to decrease amount of fat synthesized by increasing energy utilization. Certain types of damage to the area of the hypothalamus lead to excessive storage of fat.

The specific roles of hormones . . . individually and in balance with each other . . . are not clearly understood. However, their importance as "chemical regulators" in determining how the body handles fats . . . the proportion burned for energy . . . and the amount stored . . . is firmly established.

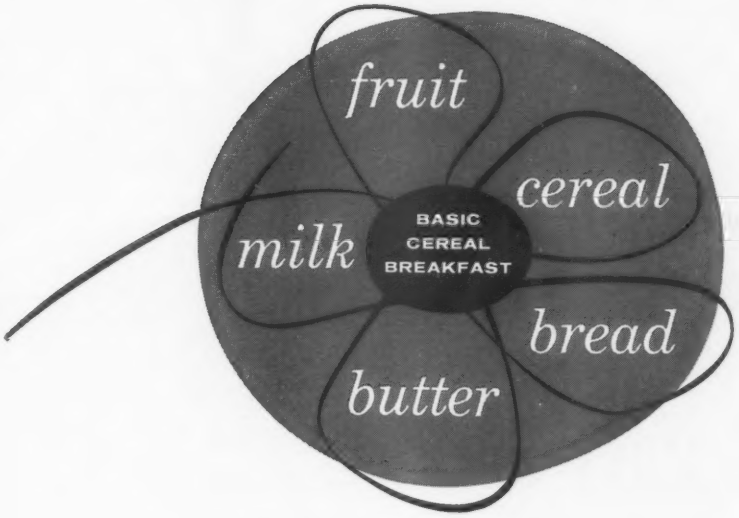
Fat, as present in foods and used in meal preparation, contributes to the pleasure of eating . . . with capacity for utilization under control of body hormones.

Since 1915 . . . promoting better health through nutrition research, education

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

NATIONAL DAIRY COUNCIL
A non-profit organization
111 N. Canal St. • Chicago 6, Ill.

THIS ADVERTISEMENT IS ONE OF A SERIES. REPRINTS ARE AVAILABLE UPON REQUEST.



this moderately *low-fat* breakfast is a well-balanced meal

When a moderate reduction of the fat calories is to be recommended in the morning meal, the basic cereal and milk breakfast merits your consideration because it is moderately low fat and contributes well-balanced nourishment as shown in the table below.

The Iowa Medical College Breakfast Studies demonstrated that this basic cereal and milk breakfast, contributing about 20 gm. high quality protein, provided quick and lasting energy during the early and late morning hours and maintained mental and physical efficiency.

basic cereal breakfast pattern

Orange juice, fresh, ½ cup,
Cereal, dry weight, 1 oz.,
with whole milk, ½ cup, and sugar, 1 tsp.,
Bread, white, 2 slices, with butter, 1 tsp.,
Milk, nonfat (skim), 1 cup,
black coffee

Nutritive value of basic cereal breakfast pattern

CALORIES	502	VITAMIN A	600 I.U.
PROTEIN	20.5 gm.	THIAMINE	0.46 mg.
FAT	11.6 gm.	RIBOFLAVIN	0.80 mg.
CARBOHYDRATE	80.7 gm.	NIACIN	3.0 mg.
CALCIUM	0.532 gm.	ASCORBIC ACID	65.5 mg.
IRON	2.7 mg.	CHOLESTEROL	32.9 mg.

Note: To further reduce fat and cholesterol use skim milk on cereal which reduces Fat Total to 7.0 gm. and Cholesterol Total to 16.8 mg. Preserves or honey as spread further reduces Fat and Cholesterol.

Bowes, A. deP., and Church, C. F.; Food Values of Portions Commonly Used. 8th ed. Philadelphia: A. deP. Bowes, 1956.
Cereal Institute, Inc.: The Nutritional Contribution of Breakfast Cereals. Chicago: Cereal Institute, Inc., 1956.
Hayes, O. B., and Rose, G. K.: Supplementary Food Composition Table. J. Am. Dietet. A. 33:26, 1957.
Cereal Institute, Inc.: A Summary of the Iowa Breakfast Studies. Chicago: Cereal Institute, Inc., 1957.

CEREAL INSTITUTE, INC. 135 South La Salle Street, Chicago 3
A research and educational endeavor devoted to the betterment of national nutrition

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THERACEBRIN

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aids in the rehabilitation of severely ill or injured patients

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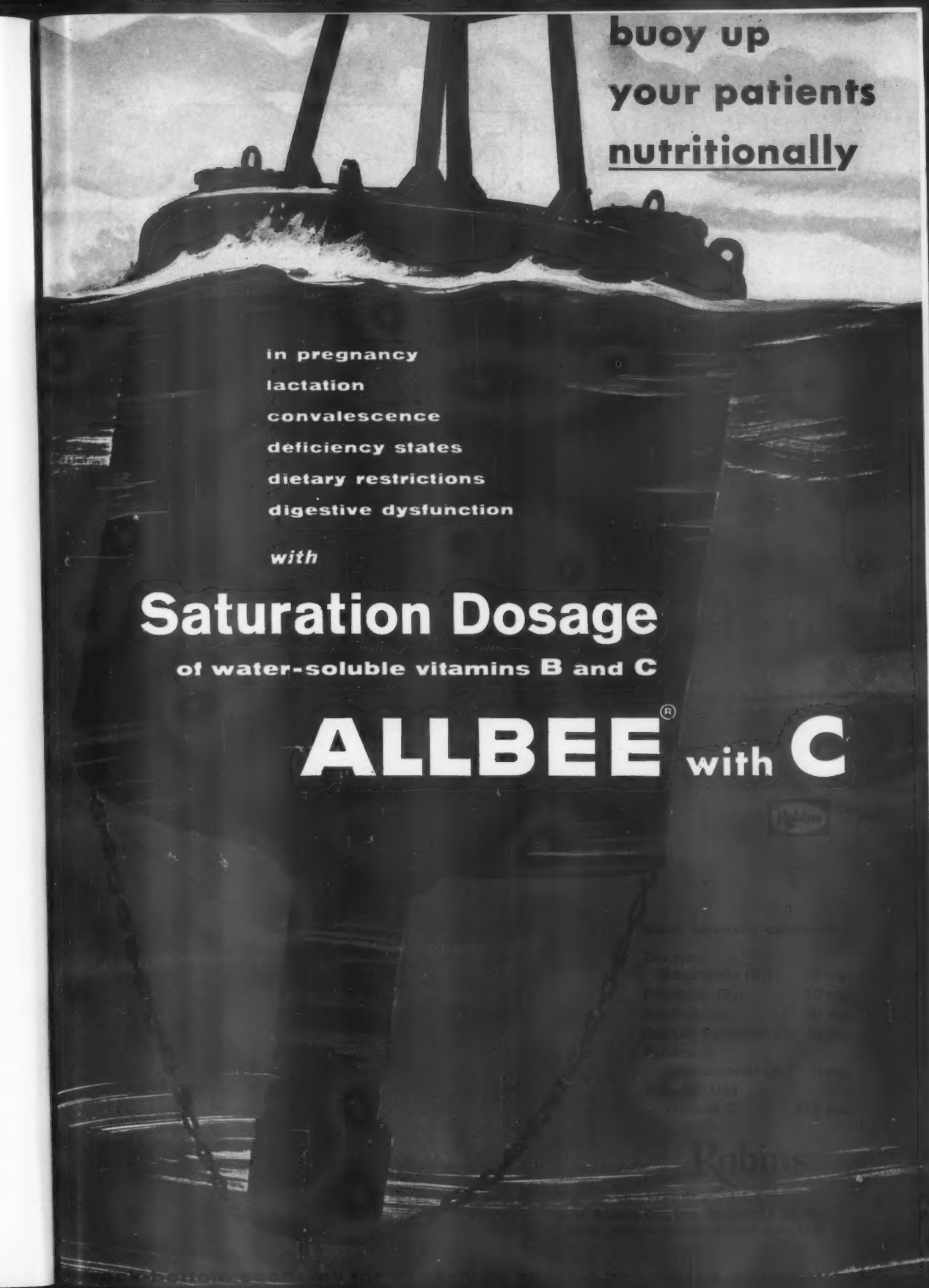
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of water-soluble vitamins B and C
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The American Journal of Clinical Nutrition

11 East 36th Street New York 16, New York

FILMS

FREE UPON REQUEST

The following list of films which are available from advertisers has been compiled from various sources. Interested readers should write to the addresses listed below for additional information—Ed.

BETTER BREAKFASTS—Three films: "*Why Eat a Good Breakfast*" for Junior and Senior High Schools; "*Skimpy and a Good Breakfast*" for Primary Grades; and "*Bill's Better Breakfast Puppet Show*" for Elementary Grades are available from State Boards of Health, Departments of Education, University Film Libraries and School Audio-Visual Departments. Preview prints may be secured from Educational Director, Cereal Institute, Inc., 135 South LaSalle Street, Chicago 3, Illinois.

IT'S ALL IN KNOWING HOW—non-technical—16 mm. sound—Kodachrome—13 1/2 minutes.

Suitable for high school and adult groups. The film uses a combination of story and animated chart techniques in presenting to teen-agers the "why and how" of good food habits. Its interest encompasses their parents as well as their teachers. Especially appropriate for science and health classes.

UNCLE JIM'S DAIRY FARM—non-technical—16 mm. sound—Kodachrome—11 minutes.

Suitable for primary and lower levels of intermediate grades. The film shows wholesome daily living on a dairy farm. It has been designed to help build ideals of healthy, happy living and emphasizes growth as a result of healthful living.

WEIGHT REDUCTION THROUGH DIET—16 mm. sound—Kodachrome—15 1/2 minutes.

Suitable for adult and professional use. The film documents a weight reduction study at Michigan State College under the direction of Dr. Margaret A. Ohlson, Head of the Foods and Nutrition Dept.

The above four films were produced by the National Dairy Council and are available for loan from: Association Films, Inc., Broad and Elm, Ridgefield, N. J.; 351 Turk St., San Francisco 2, Calif.; 561 Hillgrove Ave., La Grange, Ill.; 1108 Jackson St., Dallas 2, Texas. (There is a postage charge)

THE STORY OF OATS AND OATMEAL—16 mm. color and sound—25 1/2 minutes.

This film highlights the growth of a plant from seed to maturity. Time-lapse photography is utilized to unfold months of plant growth in seconds. The camera records crossbreeding technics used to develop the best oat strains. Dr. Frederick J. Stare, head of the Department of Nutrition, School of Public Health of Harvard University, explains the food value found in oats. The film ends with a trip through the plant of a manufacturer of oatmeal.

Film made by John Ott Pictures, Inc. for the Quaker Oats Co. It is available from the Modern Talking Picture Service, Inc., 45 Rockefeller Plaza, New York 20, N. Y.

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WINE in Geriatrics and the treatment of the Anorexic, Debilitated Patient....

From time immemorial physicians have been aware of the restorative powers of wine.

A Tasty Aid to Appetite and Digestion

A glass of Sherry at mealtime stimulates the jaded appetite, serves as a tonic and aids the digestion. As a postprandial or between-meals' beverage, a glass of Port has been warmly recommended for the sick and enfeebled.

Wine has been found to increase salivary flow and stimulate gastric secretion.

A Nutrient in Itself

The ease with which wine is metabolized makes it an important nutritive factor.

A Gentle Vasodilator and Sedative

The systemic sedative and vasodilative actions of wine can be of great aid and comfort to both the aged and the convalescent, particularly in the presence of cardiovascular disease.

These and other therapeutic uses of wine are discussed in the physician's brochure, "Uses of Wine in Medical Practice." For your free copy write—Wine Advisory Board, 717 Market Street, San Francisco 3, California.

Give your patient that extra lift with "Beminal" Forte

DURING PROLONGED ILLNESS, IN INFECTION, OR PRE- AND POSTOP

JUST ONE CAPSULE DAILY now gives therapeutic amounts of vitamin C and massive doses of B factors. Prescribe "Beminal" Forte for patients with chronic diseases and those on special diets. Where especially high vitamin B and C requirements are indicated, particularly pre- and post-operatively, 2 or 3 capsules may be given daily. (The vitamin C content was recently increased from 150 to 250 mg. with no increase in price.)

Supplied: No. 817 — Bottles of 100 and 1,000 capsules

"BEMINAL"® FORTE

Therapeutic B Factors with Vitamin C

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My formula for reinforcing primary therapy is VITERRA®

Each VITERRA Capsule contains:

Vitamins	
Vitamin A (Palmitate)	5,000 U.S.P. Units
Vitamin D (Irradiated Ergosterol)	500 U.S.P. Units
Vitamin B_{12} U.S.P.	1 mcg.
Thiamine Hydrochloride U.S.P.	3 mg.
Riboflavin U.S.P.	3 mg.
Pyridoxine Hydrochloride U.S.P.	0.5 mg.
Niacinamide U.S.P.	25 mg.
Ascorbic Acid U.S.P.	50 mg.
Calcium Pantothenate	5 mg.
Vitamin E (from mixed tocopherols concentrate)	3.7 I.U.
Minerals	
Calcium (from Dicalcium Phosphate)	213 mg.
Cobalt (from Cobaltous Sulfate)	0.1 mg.
Copper (from Cupric Sulfate)	1 mg.
Iodine (from Potassium Iodide)	0.15 mg.
Iron (from Ferrous Sulfate)	10 mg.
Manganese (from Manganous Sulfate)	1 mg.
Molybdenum (from Sodium Molybdate)	0.2 mg.
Phosphorus (from Dicalcium Phosphate)	165 mg.
Potassium (from Potassium Sulfate)	5 mg.
Zinc (from Zinc Sulfate)	1.2 mg.

the *comprehensive* nutritional supplement

In some 3 out of 4 of the patients you'll see this week, nutrition is a factor in the delicate balance between relapse and recovery.

If you back up your primary therapy with VITERRA, it will help increase the chances of therapeutic success. That's because VITERRA meets the demands of stress with both vitamins *and* minerals. You have your choice of three dosage forms: Capsules, VITERRA THERAPEUTIC (for high potencies), and VITERRA TASTITABS® (deliciously ideal for children).

Dosage: **usually one capsule daily.**

Supplied: **bottles of 30 and 100. VITERRA TASTITABS,® bottles of 100 and 250; VITERRA THERAPEUTIC,® bottles of 30 and 100.**

7256